CANADIAN JOURNAL OF AGRICULTURAL SCIENCE

(*formerly* Scientific Agriculture)

VOLUME 35 MARCH-APRIL 1955 No. 2

FERTILITY STUDIES ON SOIL TYPES

V. THE EFFECT OF CONTINUED CROPPING IN THE GREENHOUSE ON THE PHOSPHORUS SUPPLYING POWER OF SOILS[1]

L. E. Lutwick[2], A. J. MacLean[3] and R. F. Bishop[4]

Canada Department of Agriculture, Ottawa

[Received for publication September, 21 1954]

ABSTRACT

Four crops of oats and alfalfa were grown on samples of surface soil from nine farms on each of ten soil types occurring in the Ottawa district. The treatments, applied each fall at time of seeding, were 4-0-10 and 4-10-10 fertilizers at the rate of 400 lb. per acre. Yields, amounts of phosphorus removed by the crops, and available soil phosphorus estimated by Bray's methods were used to evaluate the phosphorus supplying power of the soils.

For each of the fertilizer treatments, the amounts of phosphorus taken up by the crops tended to decline with continued cropping. In most instances the response to applied phosphorus increased as cropping continued. The relative response to applied phosphorus on the different soils, however, was quite similar in the different years of cropping.

The values for acid-soluble plus adsorbed phosphorus, and for adsorbed phosphorus only, tended to decrease with continued cropping. The decline in the extracted forms of soil phosphorus relative to the amount of phosphorus removed by the crops, however, was greater in some soils than in others. Low responses to applied phosphorus were associated with high values for available phosphorus. The correlation coefficients relating the decreases in available phosphorus and the uptake of phosphorus by the crops were highly significant.

INTRODUCTION

The phosphorus status of 90 samples of surface soil, as shown by greenhouse and laboratory studies for one cropping year, was discussed in a previous publication (3). The samples were obtained from nine farms on each of 10 soil types occurring in the Ottawa district. The effect of continued cropping on the phosphorus supplying power of these samples over a 4-year period is discussed in the present paper. The yields of oats and alfalfa, the amounts of phosphorus removed by these crops, and estimates of available soil phosphorus were used to evaluate the phosphorus supplying power of the soils.

[1] Presented before Soils Section, Agricultural Institute of Canada, Macdonald College, Que., June 22, 1954. Joint contribution from the Chemistry Division, Science Service and the Field Husbandry Division, Experimental Farms Service. (Contribution No. 266, Chemistry Division).

[2] Assistant Chemist, Chemistry Division.

[3] Senior Agronomist, Field Husbandry Division.

[4] Chemist, Chemistry Division.

TABLE 1.—YIELDS OF OATS AND ALFALFA FOR THE FIRST AND FOURTH YEARS OF CROPPING WITH AND WITHOUT APPLIED PHOSPHORUS

(Mean yield per pot for 9 tests on each soil type; fertilizer applied at 400 lb. per acre)

Soil type	Oats				Alfalfa (air-dry)			
	First crop		Fourth crop		First crop		Fourth crop	
	4-0-10	4-10-10	4-0-10	4-10-10	4-0-10	4-10-10	4-0-10	4-10-10
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Uplands sand	14.8	17.6	11.3	15.1	11.6	11.9	5.7	6.2
Rubicon loamy sand	15.2	19.3	8.6	16.0	9.9	14.2	5.7	6.1
Kars gravelly sandy loam	23.1	24.3	18.1	22.4	23.7	27.0	12.9	14.4
Grenville loam	18.8	27.3	7.6	18.7	13.8	20.5	8.0	13.9
Manotick sandy loam	18.8	24.1	9.5	18.3	12.3	17.5	7.4	8.5
Castor silt loam	16.2	23.1	7.7	18.2	10.5	16.0	5.2	7.7
Osgoode loam	14.5	20.9	7.4	18.2	11.5	18.4	8.2	12.6
Carp clay loam	20.3	26.8	8.0	23.3	14.7	22.1	8.6	14.8
North Gower clay loam	18.3	25.9	8.1	18.5	13.2	21.8	8.2	15.3
Rideau clay	25.8	30.2	15.5	27.2	21.1	24.6	11.0	15.9
L.S.D. (0.05)*			4.1				3.8	

* Based on the pooled (phosphorus × farms), (years × farms) and (phosphorus × years × farms) interactions.

MATERIALS AND PROCEDURE

The greenhouse procedure given previously (3) was followed throughout the study. The 4-0-10 and 4-10-10 fertilizers, at the rate of 400 lb. per acre, were applied each fall prior to seeding the oats and alfalfa. There were three replicates for treatments in the second, third and fourth years of cropping.

The annual yields of grain, straw, and alfalfa were obtained for each pot. The phosphorus content of each of these materials was determined on composite samples from all replications according to the method of King (2).

Each year, after the final cut of alfalfa, composite soil samples were taken from the pots receiving 4-0-10 fertilizer. These, as well as the original soil samples, were analysed for adsorbed and adsorbed plus acid-soluble phosphorus by the methods of Bray (1).

RESULTS AND DISCUSSION

Yields of Oats and Alfalfa in Greenhouse

The mean yields of oats (grain plus straw) and of alfalfa, grown with and without phosphorus fertilizer, for the first and fourth years of cropping are presented for each soil type in Table 1. The yields of oats, particularly where no phosphorus was applied, and of alfalfa, regardless of treatment, tended to decrease markedly with continued cropping. The beneficial effect of applied phosphorus on oat yields was greater in the fourth than in

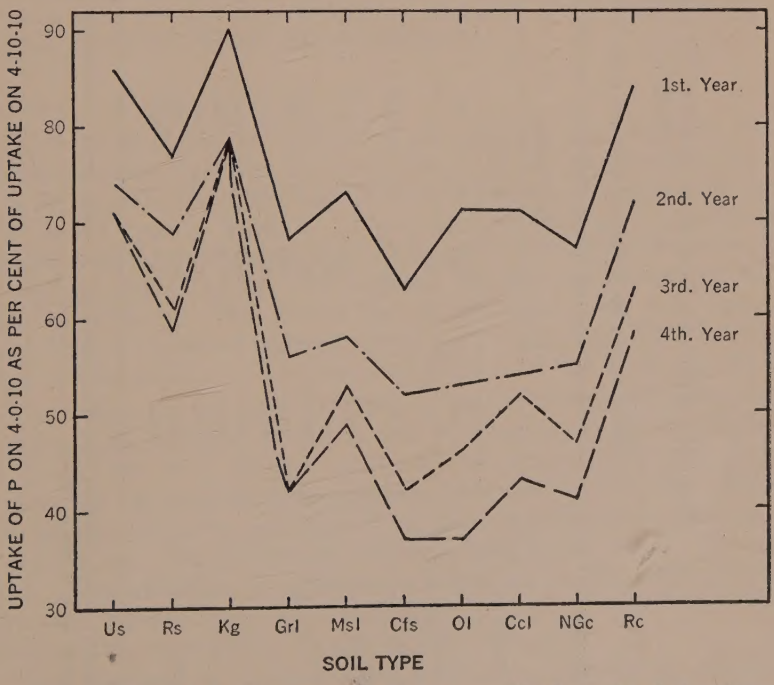

FIGURE 1. Effect of phosphorus application on combined uptake of phosphorus by oats and alfalfa in successive years of cropping.

TABLE 2.—TOTAL AMOUNTS OF PHOSPHORUS (P) REMOVED PER POT BY OATS AND ALFALFA GROWN IN THE GREENHOUSE FOR FOUR CONSECUTIVE YEARS

(Mean values for 9 tests on each soil type; 4-10-10 treatment supplied 46 mgm. P per pot)

Soil type*	4-0-10 Treatment				4-10-10 Treatment			
	Crop year				Crop year			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
Uplands	50.1	33.0	35.4	37.2	57.9	44.5	50.1	52.4
Rubicon	45.7	31.1	30.3	26.7	59.7	44.9	49.3	45.4
Kars	104.1	70.9	70.2	64.1	115.9	89.8	88.8	80.8
Grenville	49.0	29.2	23.3	19.5	71.8	51.7	55.1	46.8
Manotick	51.2	29.8	26.2	26.2	69.7	51.5	49.3	53.7
Castor	39.3	23.6	21.8	15.7	61.9	45.5	52.0	42.3
Osgoode	43.9	27.4	24.1	17.3	61.6	51.3	53.0	47.1
Carp	47.4	28.9	26.2	19.6	67.6	54.0	50.6	45.2
North Gower	45.5	34.9	24.6	18.6	68.4	63.3	52.7	45.5
Rideau	87.2	53.0	44.9	39.4	104.0	73.6	70.9	66.9

L.S.D. (0.05) = 10.7; based on the pooled (phosphorus × farms), (years × farms) and (phosphorus × years × farms) interactions.

* Series names used for sake of brevity.

TABLE 3.—ANALYSIS OF VARIANCE OF DATA FOR COMBINED UPTAKE OF PHOSPHORUS BY OATS AND ALFALFA

Source of variation	D.F.	M.S.	F
Soil type	9	16,512.26	122.97**
Phosphorus	1	84,959.96	632.71**
Years	3	21,080.55	156.99**
Phosphorus × soil type	9	430.81	3.21**
Years × soil type	27	431.93	3.22**
Phosphorus × years	3	493.05	3.67*
Phosphorus × years × soil type	27	19.57	—
Farms within soil type	80	1,751.07	13.04**
Error	560	134.28	—

* Significant at 0.05.
** Significant at 0.01.

the first cropping year. With alfalfa, however, the beneficial effect of applied phosphorus was greater in most instances in the first year of cropping. Considering depletion of nutrients in the soils as a result of continued cropping, the rate of phosphorus employed may have been too low to result in any high degree of response to phosphorus by alfalfa, particularly in the fourth year of cropping.

Phosphorus Removed by Crops

The mean values for uptake of phosphorus, as calculated from yield and composition data for the combined oat and alfalfa crops grown with the 4-0-10 and 4-10-10 treatments in four consecutive years, are presented for each soil type in Table 2. The analysis of variance in Table 3 shows that the variations in the uptake of phosphorus occurring between different soil types, between years, and between phosphorus treatments were each highly significant. The interaction of phosphorus on soil type and the interaction of years on soil type were each highly significant. The interaction of phosphorus on years was significant at the 5 per cent level.

As shown by the data in Table 2, application of phosphorus resulted in appreciable increases in the uptake of phosphorus by the crops on each soil type. For each of the fertilizer treatments there were pronounced decreases in the uptake of phosphorus between the first and second years. After the first year, the amounts of phosphorus taken up by the crops tended to decline but the differences between successive years were relatively slight. The amounts of phosphorus taken up by the crops grown on the Kars and Rideau soils were considerably higher each year than those found for the crops grown on any of the other soils.

The response to applied phosphorus, as indicated by the uptake of phosphorus on the 4-0-10 treatment expressed as a per cent of that on the

TABLE 4.—EFFECT OF CROPPING ON AMOUNTS OF PHOSPHORUS (P) EXTRACTED BY THE METHODS OF BRAY

(Means for 9 farms on each soil type; 4-0-10 treatment)

Soil type*	Acid-soluble + adsorbed P						Adsorbed P					
	Before cropping	After cropping				Total P in crops / Decline in soil P	Before cropping	After cropping				Total P in crops / Decline in soil P
		1st yr.	2nd yr.	3rd yr.	4th yr.			1st yr.	2nd yr.	3rd yr.	4th yr.	
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	mgm./p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	mgm./p.p.m.
Uplands	139	135	133	133	122	9.2	98	93	91	89	83	10.4
Rubicon	75	73	72	66	64	12.2	27	26	25	23	21	22.3
Kars	82	71	62	57	48	9.1	38	30	27	23	18	15.5
Grenville	36	32	29	27	27	13.4	12	10	9	8	8	30.3
Manotick	68	58	54	51	49	7.0	25	19	18	15	14	12.1
Castor	84	79	77	76	74	10.0	15	12	8	9	8	14.3
Osgoode	85	80	78	75	73	9.4	14	12	11	10	9	22.5
Carp	98	90	84	82	78	6.1	20	18	15	13	14	20.4
North Gower	114	106	107	103	101	9.5	19	17	17	15	15	30.9
Rideau	136	121	109	106	97	5.8	31	25	23	20	18	17.3
L.S.D. (0.05)**		5						3				

* Series names used for sake of brevity.
** L.S.D. based on the pooled (years × farms) interactions.

4-10-10, is illustrated for the different soils in Figure 1. The response to applied phosphorus increased in most instances as cropping was continued. The relative response to applied phosphorus on the different soils, however, was quite similar in the different years of cropping.

Soil Phosphorus as Determined with Methods of Bray

The effect of cropping on the amounts of phosphorus extracted from the soils is shown by the data in Table 4. The ratios of the total amount of phosphorus in the crops to the decline in soil phosphorus values as a result of cropping for a 4-year period are included in the table. The values

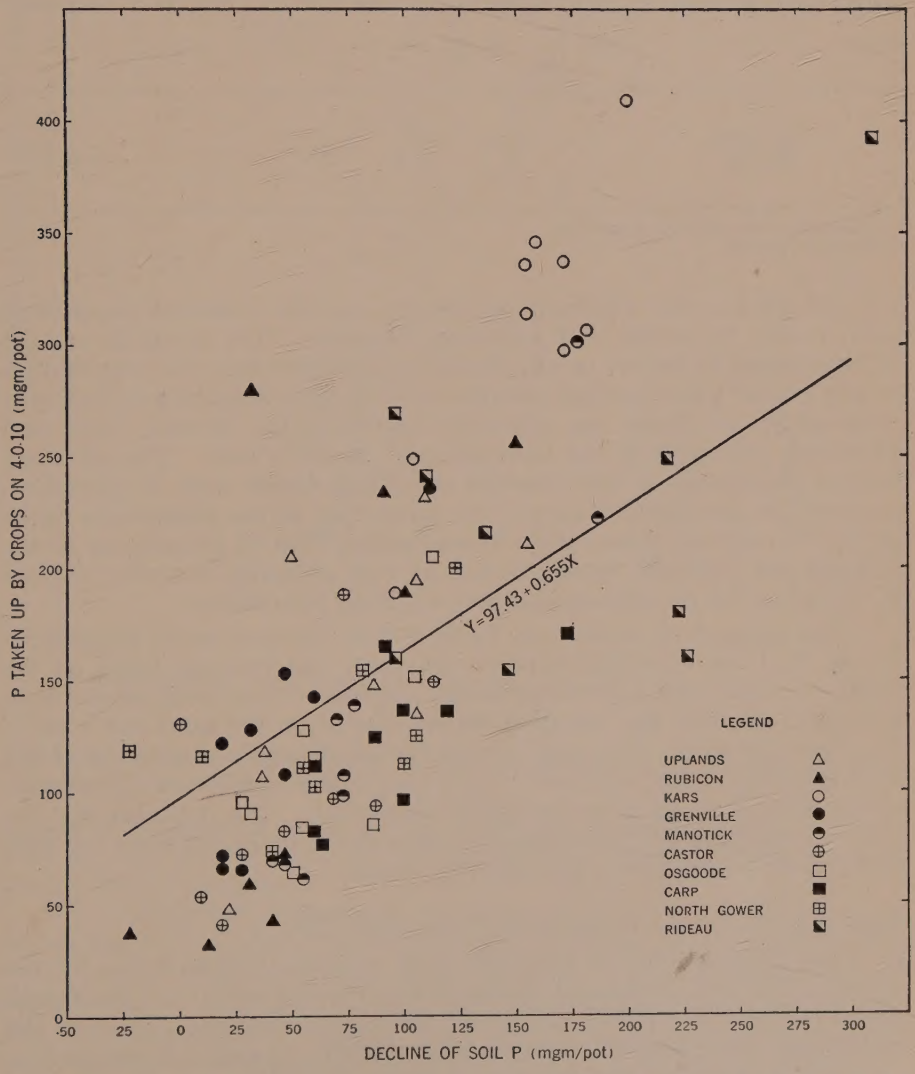

FIGURE 2. Relationship between decline of acid-soluble plus adsorbed P and amounts of P taken up by crops in a 4-year period.

TABLE 5.—ANALYSES OF VARIANCE OF DATA FOR SOIL PHOSPHORUS AS DETERMINED WITH METHODS OF BRAY

Source of variation	D.F.	Acid-soluble + adsorbed P		Adsorbed P	
		M.S.	F	M.S.	F
Soil type	9	40,390.90	1,321.69**	25,755.94	2,716.87**
Years	4	4,642.44	151.91**	1,199.94	126.58**
Soil type × years	36	153.48	5.02**	37.01	3.90**
Farms within soil type	80	8,096.81	264.95**	3,050.14	321.74**
Error	315*	30.56	—	9.48	—
$\frac{\text{Soil type}}{\text{Farms}}$			4.99**		8.44**

* Reduced by 5 because of missing values.
** Significant at 0.01.

for acid-soluble plus adsorbed phosphorus and for adsorbed phosphorus only, tended to decline with continued cropping. The ratios for uptake of phosphorus to decline in soil phosphorus indicate that the contribution of acid-soluble plus adsorbed phosphorus to the total amount of phosphorus removed by the crops was relatively greater in the Rideau, Carp, and Manotick soils than in the Grenville and Rubicon soils. The adsorbed form of phosphorus in the Grenville and North Gower soils, in particular, appeared to provide a relatively low proportion of the phosphorus taken up by the crops on these soils. The adsorbed form of phosphorus in the Uplands soil, however, tended to decline with cropping to nearly as great an extent as did the acid-soluble plus adsorbed phosphorus.

The analyses of variance in Table 5 show, for each set of phosphorus values, that the variations between soil types and between farms on the same soil type were highly significant. However, the variation between soil types exceeded the variation between farms on the same soil type at the 1 per cent level. The variations in the soil phosphorus values resulting from cropping in four successive years were highly significant. The interaction of soil type on years was highly significant. This indicates that the effect of cropping on the amounts of phosphorus extracted from the soils was greater for some soil types than for others.

Relationship of Soil Phosphorus and Greenhouse Results

The relationships between the total uptake of phosphorus by the crops on the 4-0-10 treatment for the 4-year cropping period and the decline of soil phosphorus for the same period are shown in Figures 2 and 3. The correlation coefficients expressing this relationship for each soil type and for all soil types together are presented in Table 6. Included in the table are correlation coefficients expressing the relationship between the total 4-year

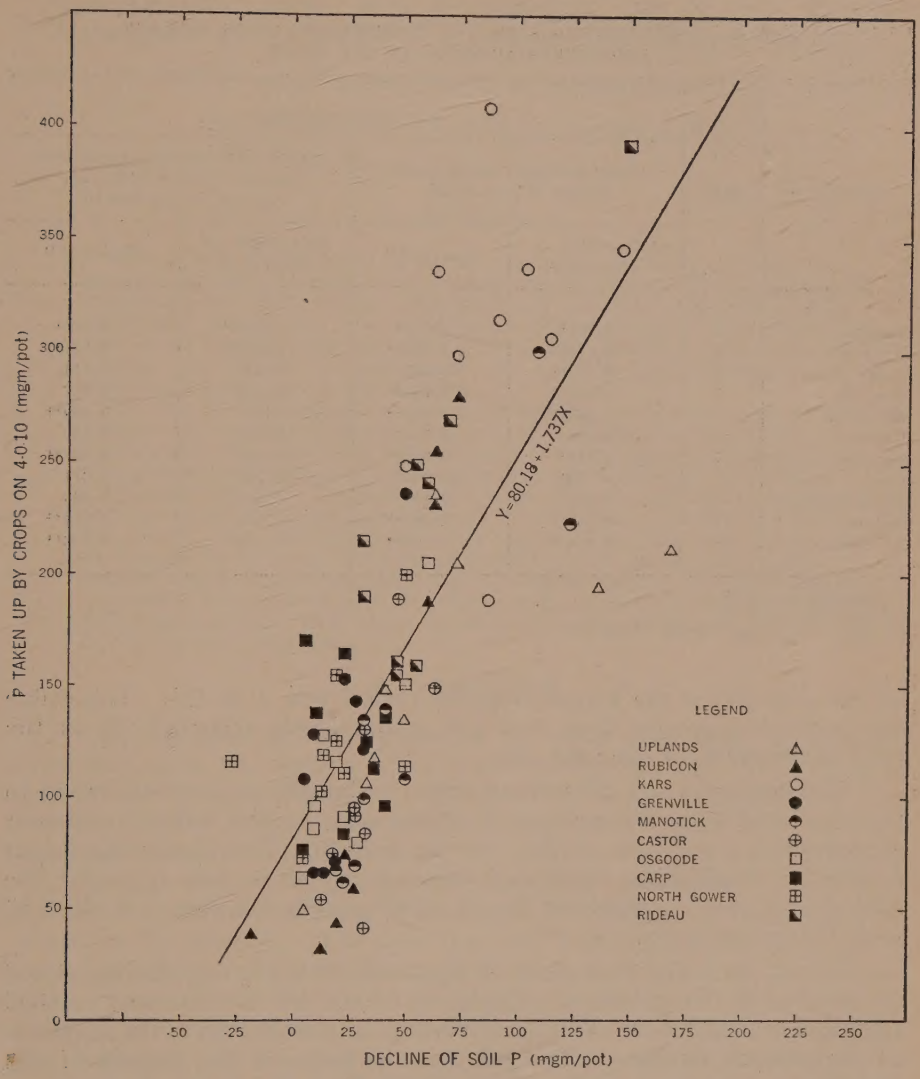

FIGURE 3. Relationship between decline of adsorbed P and amounts of P taken up by crops in a 4-year period.

uptake of phosphorus on the 4-0-10 treatment, expressed as a per cent of that on the 4-10-10, and the soil phosphorus extracted from the soil samples before cropping.

The correlation coefficients, calculated without regard to soil type, are all highly significant. The coefficients involving adsorbed phosphorus are of greater magnitude than those involving acid-soluble plus adsorbed phosphorus. It may also be noted that the deviations from the regression line involving adsorbed phosphorus (Figure 3) are not as great as those from the line involving acid-soluble plus adsorbed phosphorus (Figure 2).

The correlation coefficient showing the relationship between adsorbed phosphorus and the uptake on the 4-0-10 treatment as a per cent of that

TABLE 6.—RELATIONSHIPS OF SOIL PHOSPHORUS VALUES AND THE PHOSPHORUS REMOVED BY THE CROPS

Soil type***	D.F.	Correlation coefficients			
		Decline of soil P versus total uptake of P on 4-0-10		Soil P before cropping versus total uptake of P on 4-0-10 as per cent of that on 4-10-10	
		Acid-soluble + adsorbed P	Adsorbed P	Acid-soluble + adsorbed P	Adsorbed P
Uplands	7	0.716*	0.733*	0.815**	0.780*
Rubicon	7	0.686*	0.966**	0.903**	0.782*
Kars	7	0.879**	0.317	0.607	0.414
Grenville	7	0.898**	0.790*	0.644	0.317
Manotick	7	0.938**	0.913**	0.637	0.889**
Castor	7	0.531	0.718*	0.305	0.602
Osgoode	7	0.731*	0.845**	−0.228	0.801**
Carp	7	0.730*	−0.142	0.782*	0.371
North Gower	7	0.515	0.591	0.011	0.549
Rideau	7	0.467	0.880**	−0.042	0.801**
All soils	88	0.464**	0.753**	0.631**	0.833**

* Significant at 0.05.
** Significant at 0.01.
*** Series names used for sake of brevity.

on the 4-10-10 for the 4-year cropping period was + 0.833. This value was somewhat greater than + 0.456 as previously reported (3) on the basis of cropping for one year only.

The decline of soil phosphorus with continued cropping was found to be associated with the uptake of phosphorus on the 4-0-10 treatment expressed as a per cent of that on the 4-10-10. The highly significant correlation coefficients, calculated without regard to soil type, for the acid-soluble plus adsorbed and for adsorbed phosphorus were + 0.618 and + 0.723, respectively.

It may be noted that more of the coefficients relating decline of soil phosphorus to the uptake of phosphorus on the 4-0-10 treatment reached significance than did the coefficients relating soil phosphorus to the responses to phosphorus fertilizer. The relationship between the decline of soil phosphorus, as measured by a chemical soil test, and the amounts of phosphorus removed by the crops provides a basis for evaluating the applicability of a particular method.

REFERENCES

1. Bray, Roger H. Correlation of soil tests with crop response to added fertilizers and with fertilizer requirement. "Diagnostic Techniques for Soils and Crops", pp. 58-86. The American Potash Institute, Washington, D.C. 1948.
2. King, E. J. The colorimetric determination of phosphorus. Biochem. J. 26 : 292-297. 1932.
3. MacLean, A. J., R. F. Bishop, and L. E. Lutwick. Fertility studies on soil types. III. Phosphorus supply and requirement as shown by greenhouse studies and laboratory tests. Can. J. Agr. Sci. 33 : 330-343. 1953.

EVALUATION OF A COLORIMETRIC AND ULTRAVIOLET ABSORPTION TEST FOR DIAGNOSIS OF PLANT VIRUS DISEASES AS APPLIED TO STONE AND SMALL FRUITS[1]

N. Tomlinson and C. G. Woodbridge

Canada Department of Agriculture, Summerland, B.C.

[Received for publication October 7, 1954]

ABSTRACT

The method of Lindner *et al.* for the diagnosis and study of plant virus diseases has been investigated in connection with certain of these diseases occurring in British Columbia. Test results did not correspond with indexing of diseased cherry trees. The test failed to distinguish virus-infected cherry trees from healthy trees, cucumber plants infected with a latent virus of cherry from healthy cucumbers and virus-infected black raspberry and strawberry plants from healthy plants.

The test may be applied to distinguish healthy peach leaves from those infected with western X-disease during part of the growing season, but its specificity in this connection is uncertain. The slow spread of this virus in a tree renders adequate sampling for early detection of infection very difficult.

Evidence has been obtained which indicates that polyphenols, pentose sugars, and hydrolysis products of pentose nucleic acids are concerned in the test results. The ultraviolet absorption spectra of test solutions considered by Lindner *et al.* to be indicative of healthy plants are due principally to pentose nucleic acid hydrolysis products, while the spectra of test solutions supposedly indicative of virus infections are due principally to polyphenols and pentose sugars. Both types of solutions contain all three components, but in different proportions.

INTRODUCTION

The detection of virus infections in stone fruit trees is generally dependent upon the observation of visible symptoms. While infection by particular viruses results in the development of such symptoms in certain varieties of stone fruits, the same viruses may infect other varieties without any manifestation of symptoms (6, 18). Other viruses may give rise to symptoms in some varieties for a period of time following the initial infection, after which symptoms will no longer be visible, but the viruses will persist in the trees (5). The detection, or "indexing", of the viruses concerned in these symptomless infections is accomplished by transferring them, commonly through budding, to some suitable host tree or trees in which they do cause visible symptoms to appear. Indexing is cumbersome and time-consuming. Hence more convenient methods employing, for example, chemical techniques are desirable, particularly for use in virus disease control work and in the development of virus-free nursery stock. To be of practical value, the methods must be capable of detecting the presence of virus infection in the absence of visible symptoms, and of differentiating between infections due to different viruses.

Several chemical tests for detection of stone fruit virus infections have been recorded in the literature. In general these may be divided into two classes. The first class includes those tests in which stone fruit tree tissues are treated with dilute alkali or with alcohol and hydrochloric acid, the appearance of particular colours in the tissues or in solution following treatment indicating the presence of virus infection (10, 13, 14, 16, 17).

[1] Contribution No. 268 from the Chemistry Division, Science Service.

The second class includes tests in which the ultraviolet absorption spectra of leaf extracts or hydrolysates are determined and certain features of the spectra are considered to reveal the presence of virus infection (12, 15).

In order to evaluate the applicability of these tests in connection with the stone fruit virus diseases of British Columbia, it was decided to examine that of Lindner *et al.* (15) which incorporates the principal features of both classes.

METHOD

The method used was that of Lindner *et al.* (15). As the method is under examination, its description is included here for convenience.

In carrying out the test, very young or very old leaves should be avoided. According to Lindner *et al.*, the apparent virus content may vary from leaf to leaf and three samples should be taken from each tree. In each sample, two leaf disks $\frac{1}{4}$ inch in diameter are taken with a paper punch across the mid-vein of two leaves from the tree to be tested. The two disks are placed in a test-tube, 1 ml. of 70 per cent ethyl alcohol is added, and the tube is heated in a water bath at 80° C. for 10 minutes. The alcohol is decanted from the sample, a fresh 1 ml. portion is added, and the extraction procedure is repeated until the leaf disk is free from chlorophyll. The final alcohol wash is drained from the sample and 3 ml. of an alcohol-hydrochloric acid mixture is added (10 ml. concentrated hydrochloric acid plus 90 ml. 95 per cent ethyl alcohol). The sample is then heated at 80° C. in a water bath for 30 minutes, cooled, and the alcohol-hydrochloric acid mixture is decanted into a silica absorption cell. A Beckman DU spectrophotometer is used to take absorption readings at 2 mμ intervals in the range from 230 to 300 mμ. The alcohol-hydrochloric acid reagent is used as a reference sample. The optical density of the test solution is also determined in the visible range at 525 mμ with a Fisher Electrophotometer, the same reference sample as before being used. This latter procedure provides a measure of the intensity of the red colour, if any, of the solution.

Lindner *et al.* state that a healthy tree yields a colourless test solution having an ultraviolet absorption spectrum with an absorption maximum near 260 mμ which is characteristic of nucleic acid. A virus-infected tree yields a more or less red solution with an ultraviolet absorption spectrum characterized by high absorption at 230 mμ, a minimum between 240 and 260 mμ, and one or more maxima at or near 270 and 280 mμ. A maximum at 270 mμ is said to indicate the presence of ring spot virus disease, two maxima, one at 270 mμ the other at 280 mμ, sour cherry yellows, and two maxima, one at 274-6 mμ the other at 280-2 mμ, with or without additional maxima, western X-disease of peach.

It should be noted that where the term "healthy" has been used in describing a tree in the present work it is not necessarily synonymous with "virus-free", but indicates that the tree in question was vigorous and healthy in appearance and was free from one or more infectious agents present in any diseased tree with which it was compared. Where definite information was available regarding any latent viruses present in a tree this has been indicated in the text.

RESULTS

(*a*) *Cherry Trees*

A number of tests were made on an apparently healthy Bing cherry tree. The results are shown in Figure 1. All the test solutions were more or less red in colour, and none of their ultraviolet absorption spectra was characteristic of a healthy tree. The position of the maximum absorption in the 270 to 280 mμ region, and the intensity of the red colour as measured at 525 mμ, varied with the maturity and size of the leaf sampled. As the tree was apparently healthy, these results would have to be interpreted as indicating that the tree was infected with latent viruses. On the basis of the findings of Lindner *et al.* (15) it is difficult, if not impossible, to say what these viruses might be.

Many healthy cherry trees were examined. All gave the same general results as did the Bing tree mentioned above, with the additional finding that the position of the absorption maximum varied from about 266 mμ to 282 mμ, or did not appear at all. In the latter case the spectrum then had nearly uniform absorption in the region from 250 to between 270 and 280 mμ, with increasing absorption below 250 mμ and decreasing absorption above 270 to 280 mμ.

A few trees growing on the Experimental Station, Summerland, British Columbia, are virus-free, as judged by presently available indexing methods. These were tested, and again the results were such that they could only be interpreted as indicative of virus infection. The results included test solutions having ultraviolet absorption spectra typical of ring spot virus disease (15) which were obtained from two Mahaleb cherry trees shown to be free from ring spot infection by indexing on the test host, *Prunus serrulata* var. Shirofugen.

During the winter, cherry seedlings were grown in the greenhouse under conditions of open shading. These seedlings were partially etiolated and, when tested, yielded solutions having ultraviolet absorption spectra with an absorption maximum near 260 mμ, similar to those of a healthy tree, although the solutions showed very faint pink coloration. One flat of 24 seedlings, grown from seeds of a healthy Van cherry tree which had been indexed free from ring spot disease, was selected. One-half of the seedlings was heavily shaded, while the other half was exposed to normal daylight. At the end of one month the plants were again tested. All those exposed to sunlight now yielded pink test solutions with an ultraviolet absorption spectrum having an absorption maximum at or very near 270 mμ, whereas all the shaded plants yielded apparently colourless or faintly pink solutions, with the originally observed absorption spectrum. This result might be interpreted in two ways. The first is that a healthy cherry tree, growing under the usual conditions of light and dark, gives the test results supposedly indicative of virus infection, whereas a shaded, and consequently partially etiolated tree does not. The second is that the plants did indeed contain a virus, but that it failed to multiply under shading. The fact that the parent tree must be considered free from ring spot virus by presently accepted criteria, whereas the lighted seedlings all yielded test results indicating ring spot infection, throws doubt on the validity of the test and hence on the second interpretation of the results.

A number of tests were made of cherry trees infected with virus. Several Star and Sam cherry trees, varieties known to be infected with ring spot, were examined. All gave pink-coloured solutions having absorption spectra with a maximum at or near 280 mμ and no maximum detectable at 270 mμ, as would be expected in the case of ring spot infection. The possibility that the 270 mμ peak had been "masked" due to a very high absorption at 280 mμ, as has been suggested by Lindner *et al.* (15), cannot be accepted in this instance as by no means could the peak observed at 280 mμ be considered to reach very high proportions.

A group of Bing and Lambert cherry trees on the virus station at Summerland, which included trees infected with mottle leaf, or Lambert mottle, or both, and healthy controls was examined. Mottle leaf causes severe symptoms in the Bing variety of cherry, but is latent in the Lambert variety, while Lambert mottle causes severe symptoms in Lambert, but is latent in Bing. The two diseases have been considered to be due to distinct viruses (18, 20), but recent work has indicated that the infections in the trees tested in this group are in fact very complex*. The test results from all trees, whether healthy or diseased, were similar to those illustrated in Figure 1. No greater variation was observed between results from different trees than was observed between different tests made on a single tree. Thus, far from providing any information as to the complexity of the infections involved, which was advanced as one of the possible uses of the test (15), the results failed even to distinguish healthy control trees from diseased ones.

Similar findings have resulted from the examination of cherry trees infected with the virus diseases rasp leaf and twisted leaf found in the Okanagan Valley and little cherry found in the Kootenay Valley of British Columbia.

(*b*) *Peach Trees*

Several varieties of healthy, mature peach trees on the Experimental Station, Summerland, were tested in early September and again late in October, 1953. The results differed markedly from those secured from tests with mature cherry trees. In September the mature leaves yielded test solutions which were apparently colourless, and, while the ultraviolet absorption spectrum exhibited was not that considered by Lindner *et al.*, as indicative of a healthy tree, it did not show absorption maxima in the 270-280 mμ region of the spectrum, but rather a nearly constant absorption from approximately 250 mμ to 280 mμ, with increasing absorption below 250 mμ and decreasing absorption above 280 mμ. Immature leaves yielded test solutions with absorption spectra indicative of a healthy tree, but with pink coloration. However, when the same trees were tested again late in October, all mature leaves, whether still green or turning colour, yielded red test solutions with an ultraviolet absorption maximum at or near 280 mμ like those described previously for cherry trees and very similar to those from peach trees with western X-disease which will be discussed below. It was considered improbable that these trees had all become infected with western X-disease, but as symptoms of the disease

* Lott, T. B. *Unpublished data.* 1953.

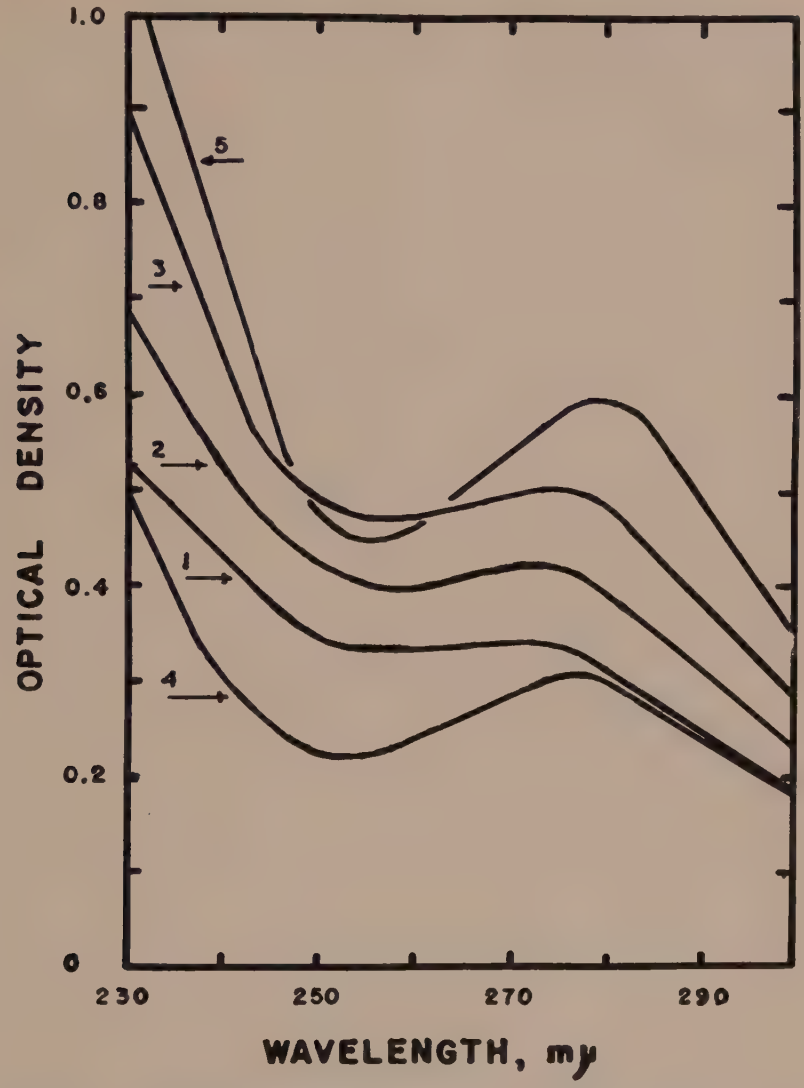

FIGURE 1. Ultraviolet absorption spectra of test solutions prepared from leaf samples from a single Bing cherry tree.

Curve	Description of leaves	Optical density of test solution at 525 mμ[1]
1	Immature, under 2 inches in length	0.17
2	Immature, under 3 inches in length	0.19
3	Mature, 4 to 5 inches in length	0.29
4	Mature, 7 inches in length	0.42
5	Mature, 7 inches in length	0.76

[1] Optical density at 525 mμ determined directly on the test solutions. Ultraviolet absorption spectra shown in curves 1, 2, and 3 determined on solutions following their dilution with an equal volume of the alcohol-hydrochloric acid mixture, those in curves 4 and 5 following dilution with two volumes.

do not necessarily appear in the first year of infection (25) the trees were re-examined for appearance of symptoms August 6, 1954. No visible symptoms of western X-disease could be detected in any of them, and it appeared that infection with the disease had not occurred. The trees were again subjected to the spectrophotometric test, and all yielded test results like those found when they were first tested in September, 1953.

Leaves from peach trees with symptoms of western X-disease located in orchards in the Oliver-Osoyoos area of British Columbia together with leaves from healthy trees in the same orchards, were tested early in September. Mature leaves from healthy trees yielded the same results as those described above from mature peach trees at Summerland in early September. Mature leaves from apparently healthy portions of infected trees yielded the same results but mature leaves from obviously diseased portions of the tree, irrespective of whether the individual leaf tested appeared normal or diseased, yielded red test solutions having ultraviolet absorption spectra with a maximum near 280 mμ similar to curve 5 of Figure 1. These spectra did not exhibit the marked irregularities reported by Lindner *et al.* (15) in connection with the disease as it occurs in Washington state.

(*c*) *A Latent Virus Disease from Cherry in Cucumber Host*

A virus disease was transmitted to cucumber (var. National Pickling) from an apparently healthy sweet cherry tree (var. Gold) which had been shown by indexing on *Prunus serrulata* var. Shirofugen to be infected with ring spot. The transmission was accomplished by grinding 1.5 gm. fresh weight of cherry flower petals in a mortar with 3 ml. of sterile distilled water (21, 22), then inoculating the cucumber cotyledons with the macerated material using the carborundum technique (24). The virus or viruses concerned in the disease transmitted have not been identified, but the symptoms produced in cucumber plants are similar to, if not identical with, those described by Boyle *et al.* (2) for a virus disease obtained from various *Prunus* species infected with either necrotic ringspot, or with necrotic ringspot and yellows.

The Gold tree from which the disease was transferred gave the same spectrophotometric test results as those described for other mature cherry trees. However, leaves from cucumber plants, whether young or mature, healthy or infected with the transmitted disease, yielded colourless test solutions with ultraviolet absorption spectra of the type described by Lindner *et al.* (15) as indicative of a healthy plant. Older cucumber leaves, whose colour was yellow-green or yellow, also yielded colourless test solutions but their maximum ultraviolet absorption was shifted from the 260 mμ wavelength found for the test solutions from younger leaves to wavelengths between 240 and 250 mμ. This capacity for absorption was the same for leaves from both healthy and diseased plants but tended to develop somewhat earlier in those from healthy plants.

(*d*) *Strawberry and Raspberry Plants*

In view of the fact that the test is said (15) to be suitable for use with some viruses of plants other than stone fruits, an examination was made of a number of strawberry and raspberry plants infected with various viruses.

The test results failed completely to distinguish between healthy strawberry plants (*Fragaria vesca*) and those infected with a mild mottle, a moderately severe mottle, leaf curl, or mild yellow-edge, or between black raspberry plants (*Rubus occidentalis*) infected with a yellow net, a mottle mosaic, or a necrotic virus, and healthy black raspberry plants. All the test solutions were apparently colourless and exhibited an ultraviolet absorption spectrum similar to that of a healthy plant as described by Lindner *et al.* (15) but with greater absorption at 230 mμ relative to that at 260 mμ.

SOME OBSERVATIONS REGARDING THE ULTRAVIOLET ABSORBING MATERIALS IN THE TEST SOLUTIONS

During the course of the above described work, investigations of the chemical composition of the test solutions were undertaken. Test solutions were prepared from young, healthy peach leaves, and from yeast nucleic acid (YNA) previously purified by the method of Smith and Markham (27). The peach test solution was apparently colourless, and had the ultraviolet absorption spectrum indicative of a healthy plant, which, according to Lindner *et al.* (15) is also characteristic of nucleic acid. However, the absorption of the solution prepared from purified YNA differed noticeably from the peach solution in the region between 230 and 240 mμ. The absorption of the former solution increased from 230 to 240 mμ while that of the latter solution decreased.

Using the two dimensional paper chromatographic technique of Carter (4) a comparison was made between these two solutions. When the two chromatograms were examined with the aid of an ultraviolet light, ultraviolet absorbing spots corresponding to the positions of guanine, adenine, guanylic acid, adenylic acid, uridylic and cytidylic acids together, uridine and/or cytidine, and a small unidentified spot near the origin were found on each. The peach chromatogram contained in addition a barely discernible amount of pink material which streaked along the direction of run of the butanol-urea solvent, but which did not move appreciably in the isoamyl alcohol-phosphate solvent. When the two solutions were mixed and run together on the same chromatogram no new ultraviolet absorbing spots were detected. Furthermore, an examination of the two solutions using the hydrolytic and paper chromatographic technique of Hershey *et al.* (8) revealed the presence of only faintly detectable traces of what appeared to be thymine in either solution, no greater proportion of this compound being present in the peach solution than was found in the purified YNA. Qualitatively, then, the test solution yielding the ultraviolet absorption of a healthy plant appeared to contain the same ultraviolet absorbing materials as did a similar solution prepared from yeast (pentose) nucleic acid, with the addition of a very small amount of some unidentified pink material.

In order to obtain information as to the ultraviolet absorption properties of this latter material, and to obtain more positive identification of the other compounds, the quantitative method of Smith and Markham (27) was used. Briefly, this method involved the preparation of paper chromatograms of healthy peach and YNA test solutions, the elution of the ultraviolet absorbing materials from the chromatograms and their examination in the ultraviolet spectrophotometer against suitable reference blanks. The chromatograms are shown in Figure 2.

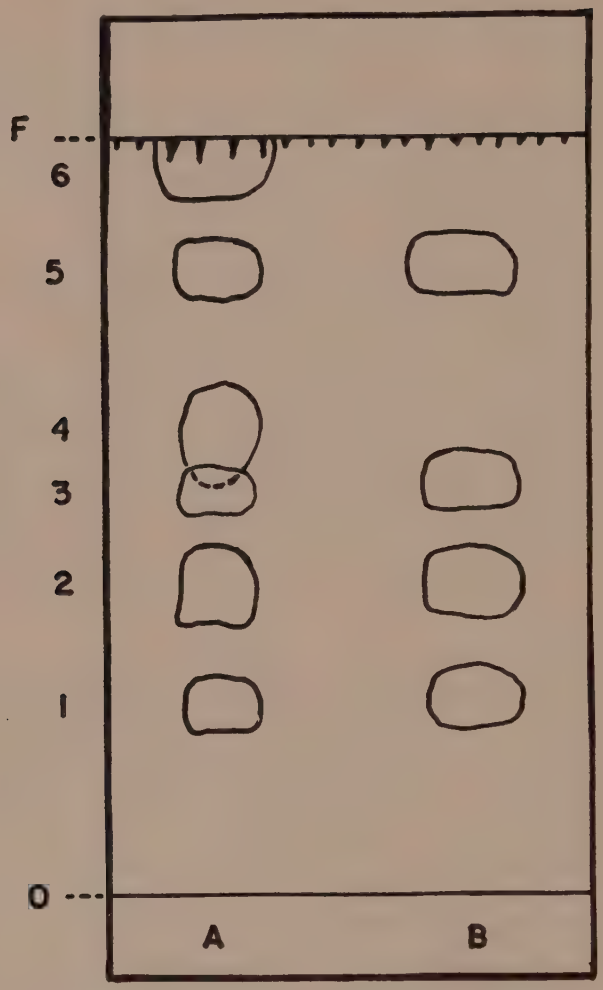

FIGURE 2. Ascending chromatogram on Whatman No. 1 paper of test solutions prepared from (A) young healthy peach leaves, and (B) purified yeast nucleic acid. Solvent system was that of Smith and Markham (27). O, origin; F, solvent front; 1, guanine; 2, adenine (possibly contains traces of guanylic acid, adenylic acid, and cytidine in addition); 3, cytidylic acid; 4, pink material; 5, uridylic acid; 6, reddish-brown material, more concentrated in darkened areas, but spread over area indicated. Yellow material always moves with the solvent front in this system, being concentrated in small spots along the front as shown.

In the preparation of the test solution from peach leaves for this purpose, several times the usual quantity of leaf tissue was employed, as a concentrated solution was desired for application to the paper. The solution thus prepared was distinctly pink in colour, although it had the same ultraviolet absorption spectrum as had the apparently colourless test solution prepared from the usual two-disk samples. Dilution of an aliquot of the concentrated solution to provide one with the same magnitude of ultraviolet absorption as had the normal test solution resulted in an apparently colourless solution. It thus appeared that the colourless test solution associated with a healthy peach actually contained a very small amount of

a pink material which, in appearance in solution, closely resembled that observed in solutions obtained previously from peaches and cherries, but found in association with an ultraviolet absorption spectrum supposedly characteristic of a virus-infected plant. Spectrophotometric measurement of eluates from the chromatograms resulted in 92 per cent of the ultraviolet absorption at 260 mμ of the YNA solution applied to the paper being recovered in guanine, adenine, cytidylic, and uridylic acids, whereas 75 per cent was recovered in these materials in the case of the solutions from healthy peach leaves, and about 10 per cent from the pink spot and the reddish brown material running with the solvent front and trailing behind it (Figure 2). No other ultraviolet absorbing materials were detected on the chromatogram of the peach solution.

Further evidence for the identification of guanine, adenine, cytidylic acid and uridylic acid from peach was obtained by comparison of the ultraviolet absorption spectrum of each of the eluates containing these materials with the corresponding eluates from yeast nucleic acid. The correspondence between the spectra was good, the only possibly significant difference being observed in the case of cytidylic acid. However, this eluate was contaminated with a small amount of the pink material in the case of peach and this may account for the observed difference. The material eluted from the pink spot of the peach chromatogram had an ultraviolet absorption spectrum in the range 230 to 300 mμ characterized by high absorption at 230 mμ, an absorption minimum near 260 mμ, and a maximum at 282 mμ above which the absorption gradually decreased. The reddish brown material moving with the solvent front and trailing somewhat behind it had a similar absorption spectrum. By combining the spectra of the various eluates from the peach solution chromatogram, a composite spectrum was obtained which resembled that of the initial solution in all important respects. The difference between the healthy peach ultraviolet absorption spectrum and that of the purified YNA, in the region between 230 and 240 mμ, was found to be due to the absorption of the coloured materials of the peach solution.

Examination by the same techniques of test solutions supposedly characteristic of virus infection obtained from cherry and peach leaves revealed that they contained materials with the same chromatographic behaviour, colour, and ultraviolet absorption characteristics as did the solution from healthy peach, but in different proportions. The coloured materials were increased and the nucleic acid hydrolysis products reduced relative to each other, the latter products sometimes being scarcely detectable on the chromatograms.

Indirect evidence for the identity of the coloured products has been obtained. When the leaf disks were heated with the alcohol-hydrochloric acid mixture it was observed that the red colour appeared most intensely along the leaf veins and then was extracted into solution. It was found that when leaf samples decolourized in the same manner as for the test were treated with aqueous ferric chloride solution blackening occurred in the regions of the leaf in which the red-coloured material was formed in the test. In addition, it was observed that in the seedling cherry leaves mentioned previously in connection with the effects of shading, the leaves

exposed to normal daylight, which gave the test indicative of virus infection, showed very heavy blackening with ferric chloride following decolorization, whereas the shaded leaves giving the apparently healthy test results showed only neglibible colour with ferric chloride. These observations pointed to a possible close relationship between the ferric chloride reactive material and that concerned in the formation of the coloured products. The ferric chloride reaction indicates the presence of polyphenols (26). Polyphenols could be concerned in the formation of the coloured products, particularly if sugars were also present (7). To test this latter possibility, leaf samples yielding the usual test results supposedly indicative of virus infection were hydrolysed for a half-hour at 80° C. with 1 N sulphuric acid. The supernatant solution was decanted from the leaf residue, carefully neutralized with ammonium hydroxide, concentrated nearly to dryness with the aid of a stream of cold air directed on the surface, and the residue extracted with 70 per cent ethyl alcohol. Aliquots of this extract were then subjected to two-dimensional paper chromatography by the method of Putnam and Hassid (23), the sheets subsequently being sprayed with *p*-anisidine hydrochloride in *n*-butanol (9). Materials were found which were indistinguishable on the chromatogram from known arabinose (large amount), xylose and ribose (lesser amounts), possible traces of glucose, and one unidentified substance.

It was found that the well-known colour-producing reactions between polyphenols and sugars (or furfurals) (7) could proceed under the conditions of the test, as the expected colours appeared when phloroglucinol or resorcinol were heated in the alcohol-hydrolchloric acid mixture with a pentose or fructose, respectively. Spectrophotometric evidence for the formation of furfurals from sugars (19) under the test conditions was also obtained, although formation was slow from the pentoses and hexoses tested with the exception of fructose.

An examination of the ultraviolet absorption of a solution of a mixture of phloroglucinol and a pentose heated together in the alcohol-hydrochloric acid mixture under the test conditions showed an absorption spectrum characterized by high absorption at 230 mμ, an absorption minimum at 250 mμ, and a maximum at 268 mμ above which wavelength the absorption once more decreased. Such a test solution, when examined by one-dimensional paper chromatography, again using the solvent of Smith and Markham (27), was found to contain two coloured materials. One was a blue substance (fluorescing red by ultraviolet light) with the same R_f as the pink material (which also fluoresces red in ultraviolet light) of the leaf extracts. The other was brown in colour and moved with the solvent front and trailed a little behind it, as did the second coloured component of the leaf extract. Spectrophotometric examination of these materials following their elution from the chromatogram showed that the material from the blue spot had an absorption spectrum characterized by high absorption at 230 mμ, an absorption minimum near 250 mμ and a maximum at 270 mμ above which wavelength the absorption once more decreased. The material from the solvent front had a similar absorption spectrum.

Thus, evidence for the presence of polyphenols and pentose sugars in decolourized stone fruit leaf tissue has been presented, and it has been

demonstrated that under the conditions of the test coloured materials are formed from polyphenols plus sugars. In addition, the coloured materials in the leaf test solutions exhibit very similar behaviour on paper chromatograms to coloured materials formed from phloroglucinol plus pentose sugars under the same conditions, but the leaf materials differ somewhat in ultraviolet absorption characteristics and in colour from the others. However, certain unidentified, soluble polyphenolic substances which occur in peach and cherry are known to exhibit an ultraviolet absorption spectrum very like that of the coloured materials of the leaf test solutions (11). This evidence favours the view that the ultraviolet absorption associated with the coloured materials formed from the leaves in the test is due to pentose sugars and unidentified polyphenols. It is the combination of this ultraviolet absorption with that of the pentose nucleic acid hydrolysis products, in different proportions, which yields the various absorption curves observed in the test results, rather than different viruses or specific products associated with the presence of particular viruses in the plant as suggested by Lindner *et al.* (15).

In explanation of the results observed with western X-disease in peach, it is suggested that the observed difference between healthy and virus-infected leaves in the test may be due to a condition in some respects akin to premature aging of the foliage of the diseased tree, a suggestion which is supported by the appearance of the foliage.

DISCUSSION

Since the original publication of the test method under examination, only one note has appeared regarding it. Blodget *et al.** observed that large variations occurred between test results from different samples from the same tree, and that there was no consistent relationship between the test results and indexing on Shirofugen. These observations are confirmed by the present findings.

In the case of western X-disease of peach, it appears that during at least a portion of the growing period the test may be capable of distinguishing between infected and healthy peach trees, although the exact specificity of the test is not known. During the first season of infection with this disease, symptoms may not appear (25), and a chemical test for infection would be of value. However, the movement of the virus in the tree is slow, possibly as little as a few inches the first year (25). Hence, in view of the evidence presented above, unless a leaf sample is taken from an infected portion of the tree the infection will not be detected. Obviously with a moderate-sized tree, adequate sampling would be tedious and perhaps impracticable. Certainly if any large number of trees were to be examined the time and labour involved would be excessive. The possible usefulness of the test in early detection of infection is consequently extremely limited.

The matter of adequate sampling for early detection of virus infection in stone fruits under field conditions may well prove to be a major difficulty in the application of any chemical tests which may be devised for the purpose. It appears that the practical usefulness of such tests might be

* Blodgett, E. C., T. O. Diener, H. E. Williams, and J. G. Barrat. *Unpublished report* of the Technical Committee of the Western Regional Stone Fruit Virus Project W-22 : 13. 1952.

limited to the examination of trees for the presence of completely systemic latent virus infections.

In view of the present findings which suggest that the same materials (polyphenols and pentose sugars) are responsible for both the colour and ultraviolet absorption spectrum of the test solutions supposedly indicative of virus infection, and of the fact that Lindner (15) considers the colour reaction in this test to be based on the same principle as that of his earlier colour tests (13, 14, 16, 17), it is perhaps not surprising that the more recent test failed, as did the earlier ones (15), to distinguish between viruses. Its inability to distinguish healthy from virus-infected cherry trees may conceivably be due to the fact that, as Lindner has suggested (17), few, if any, orchard cherry or peach trees are free of all viruses. However, to accept the results of the present test, or of any other test of uncertain specificity, in proof of this, would obviously be unsound and other conclusive evidence is not available.

The results recorded here for cucumber plants infected with a stone fruit virus are different from those recorded by Lindner *et al.* (15). They reported that when a virus was transmitted from a cherry tree infected with ringspot to cucumber, the same type of ultraviolet absorption spectrum was found for the test solutions from the infected cucumber plant and the cherry tree. In the present work, while the cherry tree yielded test solutions of the type associated with a virus disease according to Lindner *et al.* (15), the test solutions from infected cucumber plants were of the type associated with a healthy plant. The fact that the presence of the virus in cucumber cannot be detected by the test obviously does not prove that the presence of the same virus in a cherry tree cannot be detected by it, but considered in conjunction with other evidence presented it does throw additional doubt on the validity of the test.

The observation regarding the influence of shading of trees on the results of the test are similar to the earlier findings of Welsh and Wilks (28) in connection with another of Lindner's tests. The observed effect in both cases is probably due to a marked reduction in the quantity of polyphenols in the shaded leaves. It has been observed in connection with the present work that shading brings about a very large decrease in the polyphenols which are extracted from the leaves in the decolorization procedure used in the test. As certain polyphenols are known to inhibit or inactivate viruses (1, 3), and in view of the apparent impossibility of carrying out juice transfers of viruses to *Prunus* hosts (29), it seems that the use of previously heavily shaded leaves might be considered in such inoculations.

ACKNOWLEDGEMENTS

The writers wish to express their appreciation to M. F. Welsh and T. B. Lott, Plant Pathology Laboratory, Summerland, British Columbia, and R. E. Fitzpatrick, Frances C. Mellor, and R. Stace-Smith, Plant Pathology Laboratory, Vancouver, British Columbia, for very helpful co-operation in this work.

REFERENCES

1. Bawden, F. C., and A. Kleckowski. Protein precipitation and virus inactivation by extracts of strawberry plants. J. Pomol. Hort. Sci. 21 : 2-7. 1945.
2. Boyle, J. S., J. D. Moore, and G. W. Keitt. Cucumber as a plant host in stone fruit virus research. Phytopathology 44 : 303-312. 1954.
3. Carson, R. S., and A. W. Frisch. The inactivation of influenza viruses by tannic acid and related compounds. J. Bact. 66 : 572-575. 1953.
4. Carter, C. E. Paper chromatography of purine and pyrimidine derivatives of yeast ribonucleic acid. J. Am. Chem. Soc. 72 : 1466-1471. 1950.
5. Cochran, L. C., L. M. Hutchins, J. A. Milbrath, G. L. Stout, and S. M. Zeller. Ring spot. Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U.S.D.A. Agr. Handb. 10 : 71-80. 1951.
6. Harris, R. V. Plant pathology. Ann. Rept. East Malling Res. Sta. 1952. 40 : 34-38. 1953.
7. Hawk, P. B., B. L. Oser, and W. H. Summerson. Practical physiological chemistry. 12th ed. The Blakiston Company, Toronto. 1947.
8. Hershey, A. D., J. Dixon, and M. Chase. Nucleic acid economy in bacteria infected with bacteriophage T2. 1. Purine and pyrimidine composition. J. Gen. Physiol. 36 : 777-789. 1953.
9. Hough, L., J. K. N. Jones, and W. H. Wadman. Quantitative analysis of mixtures of sugars by the method of partition chromatography. Part V. Improved methods for the separation and detection of the sugars and their methylated derivatives on the paper chromatogram. J. Chem. Soc. 1702-1706. 1950.
10. Hutchins, L. M. Identification and control of the phony peach disease. Office of State Entomologist, Georgia. Bull. 78 : 1-54. 1933.
11. Johnson, G., E. M. Foreman, and M. M. Mayer. The ultraviolet absorption of polyphenolic substances from various fruits. Food Tech. 4 : 237-241. 1950.
12. Kobel, F. Zur diagnose des Steinobstvirosen. Phytopathol. Z. 20 : 353-374. 1953.
13. Lindner, R. C. A rapid chemical test for some plant virus diseases. Science 107 : 17-19. 1948.
14. Lindner, R. C., H. C. Kirkpatrick, and T. E. Weeks. A simple staining technique for detecting virus diseases in some woody plants. Science 112 : 119-120. 1950.
15. Lindner, R. C., H. C. Kirkpatrick, and T. E. Weeks. Ultraviolet absorption spectra as a tool for diagnosing plant virus diseases. Science 115 : 496-499. 1952.
16. Lindner, R. C., T. E. Weeks, and H. C. Kirkpatrick. A qualitative test for some stone fruit diseases. Phytopathology 39 : 1059-1060. 1949.
17. Lindner, R. C., T. E. Weeks, and H. C. Kirkpatrick. Studies on a colour test for stone fruit virus diseases. Phytopathology 41 : 897-902. 1951.
18. Lott, T. B. Lambert mottle. Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U.S.D.A. Agr. Handb. 10 : 123-125. 1951.
19. Low, B. Preparation of furoic acid from ribonucleic acid. Acta Chem. Scand. 4 : 294-299. 1950.
20. McLarty, H. R., T. B. Lott, J. A. Milbrath, E. L. Reeves, and S. M. Zeller. Mottle leaf. Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U.S.D.A. Agr. Handb. 10 : 106-111. 1951.
21. McWhorter, F. P. The utility of flower tissues for making inocula for difficult virus isolations. Phytopathology 43 : 479. 1953.
22. Milbrath, J. A. Transmission of components of the stone fruit latent virus complex to cowpea and cucumber from cherry flower petals. Phytopathology 43 : 479. 1953.
23. Putnam, E. W., and W. Z. Hassid. Isolation and purification of radioactive sugars by means of paper chromatography. J. Biol. Chem. 196 : 749-752. 1952.
24. Rawlings, T. E., and C. M. Tompkins. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopathology 26 : 578-587. 1936.
25. Reeves, E. L., E. C. Blodgett, T. B. Lott, J. A. Milbrath, B. L. Richards, and S. M. Zeller. Western X-disease. Virus diseases and other disorders with virus-like symptoms of stone fruits in North America. U.S.D.A. Agr. Handb. 10: 43-52. 1951.
26. Shriner, R. L., and R. C. Fuson. The systematic identification of organic compounds. John Wiley & Sons Inc., New York. 1948.
27. Smith, J. D., and R. Markham. Chromatographic studies on nucleic acids (R.N.A.). Biochem. J. 46 : 509-513. 1950.
28. Welsh, M. F., and J. M. Wilks. Induced modification of symptom severity in little cherry. Phytopathology 41 : 136-138. 1951.
29. Willison, R. S., and M. Weintraub. Studies on stone fruit viruses in cucurbit hosts. 1. A method of evaluating the infectivity of infectious juices. Phytopathology 43 : 175-177. 1953.

NECTAR SECRETION IN RELATION TO NITROGEN SUPPLY, NUTRITIONAL STATUS, AND GROWTH OF THE PLANT[1]

R. W. Shuel

Apiculture Department, Ontario Agricultural College, Guelph, Ontario

[Received for publication October 15, 1954]

ABSTRACT

Variation in growth and chemical composition of snapdragon and alsike plants growing in sand culture was effected by varying the concentration of nitrogen in the culture solution. Nectar yield per flower was comparatively good under conditions of low nitrogen supply, moderate growth, and a high level of sugar in the tissue; comparatively poor under conditions of abundant nitrogen supply, luxuriant growth, and a low sugar level in the tissue. Other sources of variation in nectar secretion were daily weather fluctuations and a nitrogen level-weather interaction. An hypothesis is advanced to account for variation in secretion in terms of the interrelationships of nitrogen supply, photosynthesis, and growth.

INTRODUCTION

In addition to its well-known service in providing honey, the honeybee makes an important contribution to the agricultural economy in cross-pollinating such crops as red clover, alsike, alfalfa, cucurbits, sunflower, and many orchard crops. Part of the pollination is accomplished by pollen-gathering bees; probably more often pollination is effected incidentally to the collecting of nectar. For the latter group, the attractiveness of the plant depends on the quantity, ease of access, and concentration of the nectar (4, 17). Pedersen (9) has found highly significant positive correlations between nectar yield and bee visitation and bee visitation and seed yields in alfalfa.

If other factors are favourable, the presence of a large population of pollinators will increase the likelihood of a good crop of seed or fruit. Any improvement in nectar production would serve to increase the pollinator population both by enhancing the attractiveness of the plant to incidental bee visitors, and, more important, by making it economically profitable to beekeepers to maintain more bees in the vicinity of the fields and orchards.

The literature on nectar secretion has recently been reviewed in detail (2) and will be discussed only briefly here. Nectar is essentially an aqueous solution of sugars whose composition varies with species, with trace amounts of other organic substances and minerals. Secretion apparently takes place as a result of metabolic activity in the nectary tissues (1, 11). Zimmermann (21) has discussed several of the theories on the significance of secretion. Liebig suggested in 1846 that sugar is excreted when it is present in the plant in large measure relative to the available nitrogen. A more recent view, held by Frey-Wyssling and others, is that secretion represents a disposal of surplus nutrients from the assimilate stream in the region of an organ whose development is terminating. The idea that nectar is an excreted surplus is common to both theories. Agthe (1) has shown that in some plants the nectar comes from the phloem sap, in others from

[1] Contribution of the Department of Apiculture, Ontario Agricultural College. The work reported here has been a part of the program of the Legume and Tree Fruit Research Committees in Ontario.

a mixture of the phloem sap and water from the xylem vessels leading to the nectary. The solute concentration of the nectar at the moment of secretion depends on the relative amounts of phloem and xylem in the vascular supply to the nectary tissue. Post-secretion changes in nectar occur rapidly and are related to atmospheric humidity (13).

There is now considerable evidence that nectar secretion is influenced in a marked degree by the quantity of carbohydrate available. Shuel (13) and Pedersen (8) found the amount of solar radiation incident on the plant during the flowering period to be important. Wykes (20) demonstrated that curtailing carbohydrate synthesis and transport to the flower by defoliation and phloem ringing, respectively, reduced nectar production in a number of species. Shuel* obtained similar results with snapdragon. Blacking-out the plant or ringing the phloem immediately below the flower spike resulted in a severe reduction in nectar yield; ringing the phloem at the base of the shoot, however, resulted in an increase in nectar production of about 33 per cent.

The present work represents an attempt to relate nectar secretion to the nutritional status and growth of the plant. Since the well-known paper of Kraus and Kraybill (7) in 1918, the significance of the relative amounts of carbohydrate and nitrogenous constituents in the regulation of plant growth has been generally appreciated. In the experiments described below, control of the nitrogen level was used as a means of effecting variation in the nutritional status of the plant.

MATERIALS AND METHODS

Plant Materials

The first plant used was snapdragon (*Antirrhinum majus* L.), previously found to be an excellent subject for nectar secretion studies. Plants of the F_1 generation of a hybrid, "Christina", were used in order to reduce genetic variability to a minimum. The study was later extended to a clonal population of alsike. All experiments were carried out in a greenhouse equipped with automatic steam valves. Thermostatic controls were set for 65° F., and night temperatures were held constant to within $\pm$ 3°. Daytime periods fluctuated considerably during sunny periods. When flowering and nectar measurement commenced, temperature fluctuations were kept to a minimum by careful manual control of the vents. Despite all precautions, some temperature variation occurred. During the snapdragon experiments, which were conducted in the winter months, temperatures in the early afternoon of bright days would sometimes rise as high as 75° for an hour or so. For the alsike experiments, done in early June, the greenhouse was sprayed with whitewash. Daytime greenhouse temperatures sometimes reached 80° (outdoor temperatures never exceeded 73°). Temperature thus constituted an uncontrolled variable whose effect on nectar secretion could not be assessed. On the basis of previous experiments with red clover, however, the effect of such short-term variation as was encountered here was felt to be of minor importance.

* Shuel, R. W. *Unpublished data.*

STUDIES WITH SNAPDRAGON

First Experiment

Fifty seedlings, about 5 inches in height, pruned to give two flower stalks, were set out in sub-irrigated sand cultures in mid-November of 1951. The irrigating solution was a modified Wagner-Pesch solution (6) made up with tap water. Each solution reservoir contained 16 litres of solution and supplied 10 plants. Solutions were changed weekly and made up to original volume with water between changes. The pH was adjusted to *ca.* 6.5 with additions of 1N HCl and 1N NaOH. The intention was to vary the nitrogen component of the solution during the later stages of growth and development. Prior to that time the nitrogen concentration was kept the same for all plants to avoid possible inter-treatment differences in flowering dates. During the pre-treatment period nitrogen was supplied as the ammonium ion to avoid the complication of nitrate accumulation, which would render close nitrogen level control during treatment impossible.

In late December, when flower stalks were beginning to elongate, plants were randomized and divided into five treatment groups. In three treatments, nitrogen was supplied at 5, 22, and 88 parts per million as $NaNO_3$; in the remaining two at 5 and 88 parts per million as NH_4Cl. All nitrogen levels were calculated to lie above the deficiency threshhold. Inequalities in osmotic pressure were corrected with additions of NaCl.

Flowering commenced in mid-January. A slight inter-treatment variation with respect to flowering dates was noticeable, flowering being earliest in the low-ammonium group and latest in the high-ammonium group. Sampling for nectar assay was begun as soon as flower production was rapid enough to provide a daily sample of at least six mature flowers from each treatment and was continued until the minimal daily flower number could no longer be obtained in the low-ammonium treatment. The individual flower rather than the plant was chosen as the sampling unit, as nectar yield per flower is the significant quantity from the standpoint of pollination. At the same time, a daily record of flower production was kept.

Nectar was extracted by centrifuging flowers in special capillary tubes calibrated for volume (16). Concentrations of total solids in the nectar were measured with an Abbé refractometer. Since sugar constitutes all but a minute fraction of the solids in nectar, the quantity "total solids concentration" will be referred to hereafter a "sugar concentration" and the quantity "weight of total solids" as "weight of nectar sugar". The latter value was derived from volume and concentration measurements. For snapdragon, total weight of sugar is a more satisfactory index of nectar production than volume and concentration values. The nectar is freely exposed in the flower to the atmosphere and undergoes rapid changes in concentration which result in wide variation in concentration readings taken on mature flowers. For this reason, concentrations have not been reported.

In addition to nectar yields, certain growth measurements are reported in Table I. The latter include plant heights, flower spike lengths, numbers of flowers harvested, and numbers of flower buds remaining at the end of the experiment.

TABLE 1.—MEAN NECTAR SUGAR YIELDS AND GROWTH DATA FOR SNAPDRAGON PLANTS UNDER DIFFERENT NITROGEN TREATMENTS, JANUARY, 1952

Treatment	Mean flowers harvested per spike	Mean residual flower buds per spike	Mean plant height (cm.)	Mean flower spike length (cm.)	Mean nectar sugar yield (mgm./flower)	Estimated potential nectar yield per plant during sampling period (mgm.)
p.p.m. Nitrogen as:						
Nitrate						
88	8.3	3.5	82.7	16.5	1.03	17.1
22	9.6	3.9	74.3	13.0	1.05	20.2
5	6.3	3.7	72.5	11.0	1.24	15.6
Ammonium						
88	8.2	4.8	74.8	12.5	0.94	15.4
5	7.1	1.6	67.2	10.3	1.32	18.7
L.S.D.—						
5 per cent level	—	—	8.9	2.6	0.17	—
1 per cent level	—	—	11.9	3.5	0.22	—

Results: Plant growth was clearly related to the concentration of nitrogen in the irrigating solution. Plants supplied with a high level of nitrogen had darker, more luxuriant foliage, were taller, and had longer flower spikes with more flowers than plants supplied with less nitrogen. Wadleigh (19) noted a similar relationship between growth of cotton and concentration of nitrogen in the culture solution. The trend in nectar production, calculated on a flower basis, was the opposite to that of growth; flowers from low-nitrogen treatments, fewer in number, yielded considerably more nectar. The form in which nitrogen was supplied, whether as a nitrate or an ammonium salt, made no perceptible difference in nectar yield.

It can be seen from Table 1 that more flowers were produced in the high-nitrogen treatments. For this reason the estimated potential yield of nectar per plant for the sampling period (mean yield per flower $\times$ mean flower number) was higher in some of the high-nitrogen groups. As inter-treatment differences in flower production were not constant from day to day, it was possible to assess the data for a relationship between daily flower production and nectar yield per flower. The two quantities appeared to be completely independent. Yield per flower was therefore retained as the chief criterion of nectar production.

In addition to treatment differences in nectar yield, a highly significant interaction between nitrogen level and daily weather conditions occurred. The relationship of nectar yield to solar radiation, the chief weather variable under the conditions of the experiment, is illustrated in Figure 1 (*a*). Values for daily solar radiation, expressed as gram calories per cm.^{2}, and representing an integration of intensity and duration of sunlight, were furnished by the Air Services Meteorological Division of the Department of Transport. These values were calculated from the observed daily radiation at Toronto and the number of hours' sunshine at Toronto and Guelph, and are accurate on the average to within about $\pm$ 15 per cent.

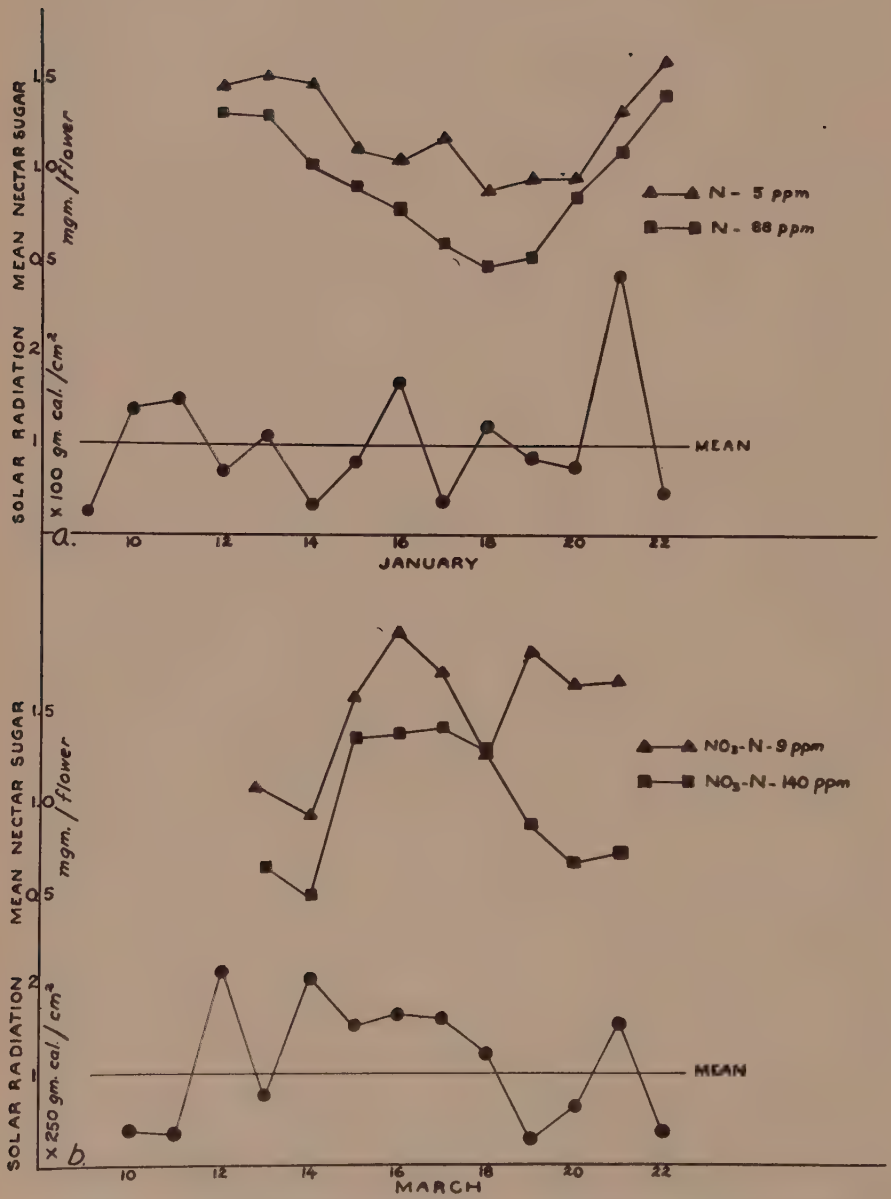

FIGURE 1. The relationship of nectar yield in snapdragon to solar radiation. Flowers were harvested daily at 2 p.m. in January, at 10 a.m. in March.

Second Experiment

Plants were now cut back and returned to the ammonium source of nitrogen until elongation of flowering stalks commenced in late February. Treatment differences were then imposed.

The same plants were used with each treatment as before. Treatments were similar to those in the first experiment except that all nitrogen levels were raised by about 60 per cent to compensate for the normal seasonal increase in solar radiation. From March 13 to March 21 flowers were collected daily for nectar assay. Nine flowers were taken from each treatment group. Measurements of plant height, spike length, and flower number were again made and are listed in Table 2 with nectar yield data.

On March 22, the plant tops were cut off, weighed, and prepared for chemical analysis. Composite samples were made of all the plants within each treatment. The freshly harvested material was heated in an air blast at 77° C. for one hour, then at 62° C. for several hours. It was next ground in a Wiley mill and dried to constant weight in a vacuum oven at 60° C. The material was then analysed for various nitrogenous and carbohydrate constituents. Owing to the large number of analyses carried out and the paucity of material some determinations were made on a semi-micro basis.

Total nitrogen less nitrate was determined by a micro-Kjeldahl method (5), nitrate nitrogen by the phenoldisulphonic acid method (12). Total nitrogen was calculated as the sum of the values for these two determinations. As some of the nitrate was probably included in the Kjeldahl measurement, the values obtained for total nitrogen were most likely high. For amide nitrogen the extraction method of Steward and Street (15) was used, followed by analysis according to the method of

TABLE 2.—MEAN NECTAR SUGAR YIELDS AND GROWTH DATA FOR SNAPDRAGON PLANTS UNDER DIFFERENT NITROGEN TREATMENTS, MARCH, 1952

Treatment	Mean flowers harvested per spike	Mean residual flower per spike	Mean plant height (cm.)	Mean spike length (cm.)	Mean fresh weight (gm.)	Mean nectar sugar yield (mgm./ flower)	Estimated potential nectar yield per plant during sampling period (mgm.)
p.p.m.							
Nitrogen as:							
Nitrate							
140	9.1	8.0	77.8	20.0	99.8	0.98	17.8
35	9.2	5.2	76.5	17.6	74.4	1.42	26.1
9	9.1	5.3	68.4	14.2	55.0	1.51	27.4
Ammonium							
140	8.1	5.4	60.2	10.9	44.7	1.38	22.3
9	9.0	3.6	65.0	13.6	53.3	1.42	25.6
L.S.D.—5 per cent level			7.9	2.8	15.4	0.14	
1 per cent level			10.7	3.6	20.7	0.19	

TABLE 3.—EFFECT OF NITROGEN TREATMENT ON CHEMICAL COMPOSITION OF SNAPDRAGON TOPS, MARCH, 1952

Treatment	Per cent moisture	Nitrate N	Amino acid and amide N	Ammonia N	Total N	Reducing sugars	Sucrose	Starch
	(All values expressed as per cent of oven-dry weight)							
p.p.m.Nitrogen as: Nitrate								
140	82.9	0.60	0.158	0.004	4.10	1.60	0.54	0
35	80.6	0.10	0.109	0.003	2.30	2.57	0.37	0.87
9	78.9	0.05	0.106	0.003	1.70	3.18	0.21	4.19
Ammonium								
140	79.2	0.10	0.175	0.011	3.90	2.89	0.36	0.15
9	77.7	0.05	0.120	0.003	1.95	3.25	0.44	3.03
L.S.D. at 5 per cent level	—	0.18	—	—	0.23	0.21	—	0.23
	—	0.24	—	—	0.34	0.29	—	0.34

Pucher, Vickery, and Leavenworth (10). The extract prepared for amide nitrogen analysis was used in determining free amino acids by the ninhydrin method of Van Slyke *et al.* (18).

For sugar analyses, extraction was carried out according to Browne and Zerban (3), except that a 6-hour period of refluxing was substituted for the 12-hour Soxhlet extraction. Aliquots of the extract were analysed for reducing sugars by the Somogyi method (14) before and after inversion by the method of Shapiro (3). Sucrose was calculated from the difference between the two results. A starch determination was made on the residue from the sugar extraction, using the method of Sullivan (3).

Results: The trends in growth and nectar production with treatment were similar to those observed in the earlier experiment except for the plants in the high-ammonium treatment. Here, despite the high concentration of nitrogen in the irrigating solution and dark-green foliage indicative of an abundance of nitrogen in the plant, growth was poor and nectar yield was high. As before, significant daily variations and treatment-day interactions in nectar yield appeared [Figure 1 (*b*)]. Again flower production was more abundant in the high nitrogen (nitrate) treatment. The number of flowers produced during the sampling period, however, was fairly uniform among the different groups and the order of nectar yield was the same whether calculated on a per flower or a per plant basis. No relationship between daily flower number and nectar yield per flower was evident.

The results of the chemical analyses of plant shoot tissue appear in Table 3. The total nitrogen content of the tissue bore a direct relationship to the concentration of nitrogen in the culture solution. Considerable variation was evident in the individual nitrogenous components. Nitrate was accumulated in plants of all treatments (apparently from traces of nitrate in the tap water used in making up the solutions, in the case of the ammonium series), but in a marked degree only in the high-nitrate plants. High-nitrogen plants had considerably more amino acid and amide nitrogen than low-nitrogen treatments. A conspicuous accumulation of ammonia took place in the high-ammonium plants. The poor growth of this group may have been due to a toxic condition resulting from this accumulation. Carbohydrate fractions were present in inverse proportion to the nitrogenous components except in the case of sucrose, which varied little among groups. Very little starch was accumulated in the plants supplied with the higher levels of nitrogen. The content of reducing sugar in the high-ammonium treatment was quite large compared with the highest nitrate-nitrogen group. Chemical composition and nectar yield are related graphically in Figure 2.

ALSIKE STUDIES

As normal populations of clover are extremely heterogeneous with respect to nectar production, vegetatively propagated plants were used in these studies. In the winter of 1952-53 a clone of alsike was established by means of crown cuttings and grown in sterilized sand to which was added periodically a mineral solution of the following composition, made up with distilled water: NH_4Cl, 215 mgm.; K_2SO_4, 210 mgm.; $MgSO_4 \cdot 7H_2O$, 495 mgm.; $NaH_2PO_4 \cdot H_2O$, 140 mgm.; and $CaCl_2 \cdot 2H_2O$, 145 mgm. per

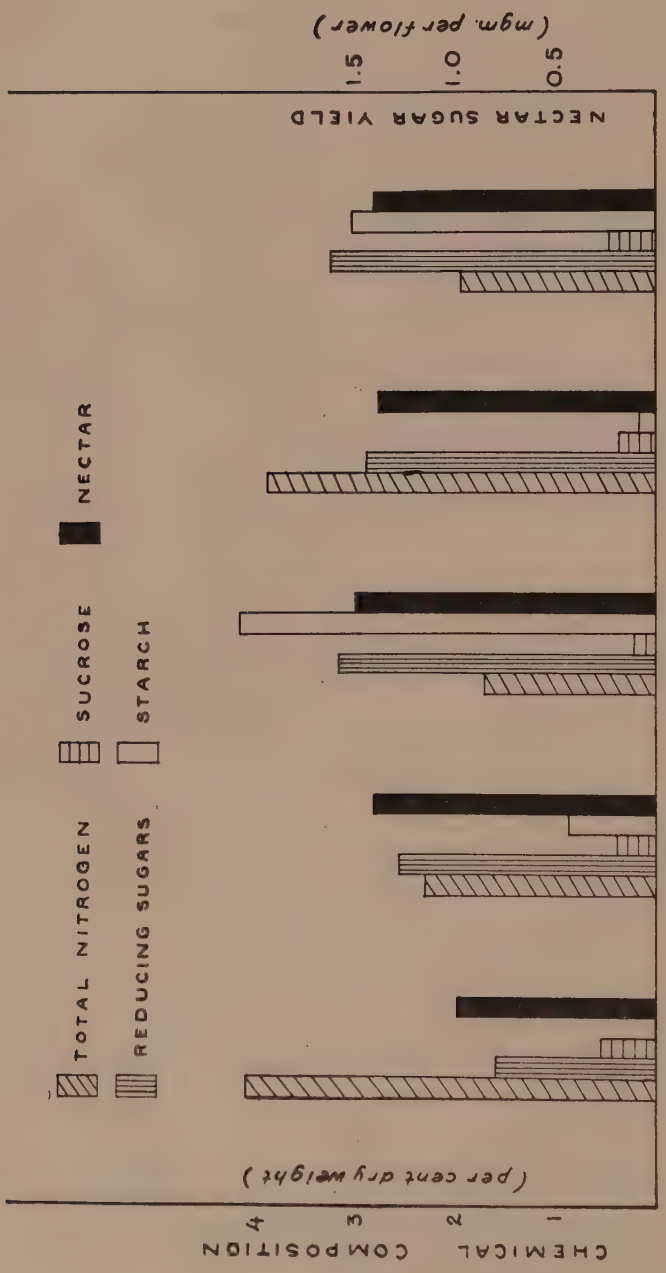

FIGURE 2. The relationship of nectar yield in snapdragon to the level of various nitrogenous and carbohydrate constituents of the plant tissue. The level and form of nitrogen supplied in the culture solution is indicated at the bottom of the chart.

litre of solution, plus small amounts of iron as ferric tartrate and the essential trace elements. In the latter part of April the plants were transferred to special containers and irrigated daily with slightly modified versions of the solution described above. Nitrogen was now supplied, as $NaNO_3$, at 2 levels: 192 p.p.m. (High) and 38 p.p.m. (Low).

The alsike flowered in early June. Twelve flowers from each treatment were assessed for nectar on each of six days. In addition to the nectar data, inflorescence number, inflorescence weights, and weights of plant tops were recorded. Tops were then dried and ground and analysed by the Department of Chemistry, Ontario Agricultural College, for total nitrogen, reducing sugars, sucrose, and starch. Data on alsike are reported in Table 4. Nectar concentrations are reported as well as sugar yields. Nectar concentration is much less variable in alsike than in snapdragon, probably because the exposed nectar surface is much smaller.

Basis for Comparison of Alsike Nectar Yields

As each floret in an alsike head is a potential nectar producer, nectar yield is best calculated on a floret basis. Counting of florets is extremely time-consuming, however, and in view of the fairly good correlation existing between floret number and head weight, floret counts were dispensed with and heads were weighed instead. Although treatment mean head weights for the whole sampling period were almost identical, variation from day to day was considerable. Nectar yields were, therefore, adjusted for regression on head weight by the co-variance method. This type of adjustment was deemed preferable to a simple arithmetical correction because the correlation between floret number and head weight was not perfect. Both adjusted and unadjusted mean yields are included in Table 4; actually there is little difference between the two sets of means. The co-variance adjustment, however, made considerable difference in mean daily yield values.

Results: In spite of the precautions taken against the establishment of nodules, some root nodulation occurred in all plants. As a result, the effect of differences in nitrogen level in the substrate on plant composition was probably less pronounced than it would otherwise have been. As it was, chemical analysis revealed a higher nitrogen content in the high-nitrogen group. Differences in weight between the two groups fell slightly short of statistical significance. There was no appreciable difference in flower number. Low-nitrogen plants had a higher content of reducing sugars and sucrose but a slightly lower starch content.

Nectar sugar yield was substantially higher in the low-nitrogen group. The daily variation was highly significant statistically, but the treatment-day interaction was non-significant. The relation of daily mean nectar yield to solar radiation is illustrated in Figure 3. Solar radiation data are from recording potentiometer records at the Physics Department, Ontario Agricultural College. Because of the whitewash on the greenhouse the quantity of radiation reaching the plants was less than the recorded values. Relative daily radiation, however, is accurately portrayed in the graph in terms of the mean for the 6-day period.

TABLE 4.—NECTAR YIELD, GROWTH MEASUREMENTS, AND CHEMICAL COMPOSITION OF ALSIKE AT TWO LEVELS OF NITRATE NITROGEN, JUNE, 1953

Nitrogen level	Plant fresh weight (gm.)	Per cent moisture	Mean head number	Mean head weight (mgm.)	Mean nectar sugar field (mgm. head)		Mean nectar concn. (per cent)	Chemical composition (per cent oven-dry weight)			
					unadjusted	adjusted		Total N	Reducing sugar	Sucrose	Starch
High	439	19.9	238	331	6.48*	6.40**	58.3**	2.15	4.06	1.63	12.2
Low	378	20.9	217	326	7.48	7.57	61.4	1.82	4.37	1.76	11.6

* Mean difference significant at 5 per cent level.
** Mean difference significant at 1 per cent level.

Nectar concentration was influenced by nitrogen level, weather factors, and a treatment-weather interaction. Nectar from the low-nitrogen plants was the more concentrated, its superiority being greatest on days with the least sunshine.

DISCUSSION AND CONCLUSIONS

Three known sources contributed to variation in nectar yield in the snapdragon and alsike studies: nitrogen level, daily weather fluctuations, and the interaction between nitrogen level and weather.

Data from chemical analyses of the plant tissue can be used in drawing inferences with respect to the first source of variation only. The values obtained for the various constituents are a measure of the average chemical composition of the plants in the various treatment groups at the end of the experimental period. It would, of course, have been desirable to have obtained analytical data on a daily basis, as some variation in composition would be expected from day to day and even in the course of a single day. The present data should, however, be a fairly reliable index of average inter-treatment differences in the various fractions for the whole of the flowering period.

It is apparent from a consideration of Tables 2, 3 and 4, and Figure 2, that nectar production was highest in the plants whose tissues had the most sugar. In Figure 2 nectar production in snapdragon can be seen to parallel reducing sugar level, and in Table 4 it can be seen that the higher-yielding group of alsike plants had the greater sugar content. No distinct proportionality between nectar production and tissue content of any of the other components analysed can be seen. Sugar level was, in general, inversely correlated with nitrogen content, which in its turn was directly related to the concentration of nitrogen in the culture solution. An anomalous situation existed in the high-ammonium group of snapdragons, in which both sugars and nitrogenous components were abundant. Here growth was checked by an unidentified factor, possibly an ammonia toxicity.

Nectar yield per flower was comparatively poor in plants of luxuriant growth and comparatively good in plants of meagre growth. Relative performance varied somewhat with weather conditions.

The extent of daily variation in nectar production can be seen in Figures 1 and 3. Fluctuations in the chief weather variable, solar radiation, are shown. In alsike (Figure 3) a close positive relationship between nectar yield and the quantity of solar radiation in the 24-hour period immediately preceding harvest ($r = +0.953$) can be seen. A similar relationship has been found in red clover (13). In the case of the snapdragons, the association is not so clear-cut. Here secretion commences about three days before the flower reaches maturity. The size of the yield, therefore, will be a function of the aggregate solar radiation over a period of several days, although the day prior to harvest is probably the most critical. In any event, nectar yields were obviously higher during periods of sunny weather.

Some aspects of the nature of the interaction of nitrogen level with weather conditions in nectar production may be inferred from the graphs in Figure 1. Although low-nitrogen plants were superior as nectar pro-

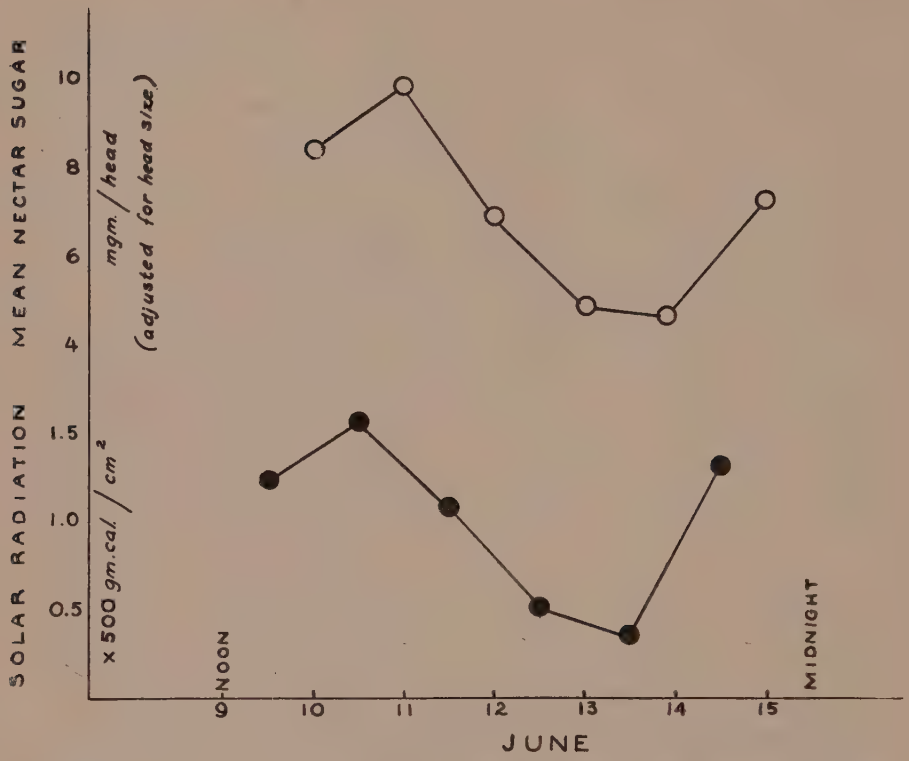

FIGURE 3. The relationship of nectar yield in alsike to solar radiation in the 24-hour period immediately preceding harvest. The coefficient of correlation, $r = +0.953$, was highly significant statistically. Flowers were harvested at 2 p.m. each day.

ducers, their relative superiority was not consistent from day to day. In general, the greatest differences between the low-nitrogen and high-nitrogen groups occurred on days when all nectar yields were low, during or immediately following a period of little sunshine. No significant treatment-day interaction with respect to total nectar sugar was found in alsike, but a well-defined interaction was found for nectar concentration. The degree of superiority of low-nitrogen plants was closely and negatively correlated with solar radiation ($r = -0.887$). A cause-effect relationship cannot be demonstrated, however, as atmospheric humidity also varied during this period, concurrently with, but in the opposite direction to, solar radiation. Moreover, it is not known whether concentration differences arose during or following secretion.

A possible explanation of the variation in nectar yield in the present experiments, consistent with the observations listed above, is as follows: Within the limits imposed by heredity, the quantity of nectar secreted was in large part dependent on the supply in the plant of the recent products of photosynthesis. In this instance, the level of reducing sugars, or substances in equilibrium with reducing sugars, appears to have been critical. As these substances are also utilized in growth, respiration, and other vital processes, the stock available for secretion would depend not only on the

amount synthesized but also on its partition among various processes. Nectar was generally more abundant in sunny weather because more sugars were synthesized then. The proportion of these products going into nectar production would, however, be conditioned by the nitrogen status of the plant. In the absence of other limiting factors, the amount of growth which occurred depended on the abundance of nitrogen in the plant, which in turn was a function of the nitrogen supply to the root system. In plants with a low level of nitrogen a nitrogen deficit would limit growth, and a surplus of carbohydrates would accumulate. Under such conditions nectar secretion would be relatively copious. In plants receiving an abundant supply of nitrogen, on the other hand, more of the products of photosynthesis would be drawn into protein synthesis and growth, and carbohydrates would not accumulate to the same extent. There would still, however, be a sufficient supply of sugars to provide for moderately good nectar secretion. In addition, the more extensive leaf surface of the high-nitrogen plants should have supported more photosynthesis and partially offset the advantage of the low-nitrogen plants with respect to nectar secretion.

During cloudy weather the carbohydrate supply (aside from storage forms) would be smaller. In plants with excessive nitrogen, most of the free sugars would be utilized in growth and related processes and nectar secretion would be poor. In plants with comparatively little nitrogen the relative sugar deficit would be less acute, and moderately good nectar production could still take place.

This scheme is, of course, oversimplified. Factors other than sugar level must have been operative in imposing limits on nectar secretion. High-nitrogen plants secreted some nectar even in cloudy weather. Furthermore, as nectar secretion is restricted to very small areas in the plant, the nectaries, it is probable that local conditions, which may differ considerably in degree from general conditions, are important. The question of sugar transport probably enters here. Nor can the hypothesis be applied *in toto* to nectar production in orchard crops which flower before the leaves expand; here the nectar must arise from storage products rather than from recently elaborated carbohydrates. However, the argument with respect to the carbohydrate-nitrogen balance should still be valid.

The data obtained in these experiments are consistent with Liebig's view that excretion of sugar occurs when sugar is over-abundant relative to nitrogen and also with the view of Frey-Wyssling and others that it is a disposal of surplus nutrients from the assimilate stream in the region of a developing organ. The two theories are, in fact, not incompatible, as the former is a statement in terms of the general nutritional status of the plant, while the latter takes cognizance of local conditions.

In terms of seed or fruit production, a level of nitrogen favouring excessively vigorous vegetative growth might be expected to produce plants lacking in attractiveness to insect pollinators because of low nectar yield. This factor may contribute to the poor seed yields observed in such plants (8).

ACKNOWLEDGEMENTS

The author wishes to thank Robert Slinger and members of the Chemistry Department, Ontario Agricultural College, for undertaking the chemical analyses reported here; Andrew Thomson, of the Meteorological Division, Department of Transport, and Robert Gage, Physics Department, Ontario Agricultural College, for providing weather data; and Hugo Barrales for technical assistance.

REFERENCES

1. Agthe, C. Ueber die physiologische Herkunft des Pflanzennektars. Promotionsarbeit E.T.H., Zurich, 2017 : 240-274. 1951.
2. Beutler, Ruth. Nectar. Bee World 34 : 106-116, 128-136, 156-162. 1953.
3. Browne, C. A., and F. W. Zerban. Physical and chemical methods of sugar analysis. 3rd ed. John Wiley & Sons, New York. 1941.
4. Butler, C. G. A study of the frequency with which honeybees visit red clover (*Trifolium pratense* L.) together with an examination of the environmental conditions. Ann. Appl. Biol. 28 : 125-134. 1941.
5. Campbell, Walter R., and Marion I. Hanna. The determination of nitrogen by modified Kjeldahl methods. J. Biol. Chem. 119 : 1-7. 1937.
6. Kiplinger, D. C., and A. Laurie. Growing ornamental greenhouse plants in gravel culture. Ohio Agr. Expt. Sta. Bull. 634. 1942.
7. Kraus, E. J., and H. R. Kraybill. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Expt. Sta. Bull. 149. 1918.
8. Pedersen, M. W. Experimental factors affecting nectar secretion and seed production in alfalfa. Agron. J. 45 : 359-361. 1953.
9. Pedersen, M. W. Seed production in alfalfa as related to nectar production and honeybee visitation. Bot. Gaz. 115 : 129-138. 1953.
10. Pucher, George W., Herbert B. Vickery, and Charles S. Leavenworth. Determination of ammonia and of amide nitrogen in plant tissue. Ind. and Eng. Chem. 7 : 152-156. 1935.
11. Radtke, F. Anatomische-physiologische Untersuchungen von Bluetennektarien. Planta 1 : 389-418. 1926.
12. Roller, E. M., and N. McKaig, Jr. Some critical studies of the phenoldisulfonic acid method for the determination of nitrates. Soil Sci. 47 : 397-407. 1939.
13. Shuel, R. W. Some factors affecting nectar secretion in red clover. Plant Physiol. 27 : 95-110. 1952.
14. Somogyi, Michael. A new reagent for the determination of sugars. J. Biol. Chem. 160 : 61-68. 1945.
15. Steward, F. C., and H. E. Street. The soluble nitrogen fractions of potato tubers: the amides. Plant Physiol. 21 : 155-193. 1946.
16. Swanson, C. A., and R. W. Shuel. The centrifuge method for determining nectar yield. Plant Physiol. 25 : 513-520. 1950.
17. Vansell, George H. Relation between the nectar concentration in fruit blossoms and the visits of honeybees. J. Econ. Ent. 27 : 943-945. 1934.
18. Van Slyke, D. D., D. A. MacFayden, and D. Hamilton. Determination of free amino acids by titration of the carbon dioxide formed in the reaction with ninhydrin. J. Biol. Chem. 141 : 671-680. 1941.
19. Wadleigh, C. H. Growth status of the cotton plant as influenced by nitrogen supply. Arkansas Agr. Expt. Sta. Bull. 446. 1944.
20. Wykes, G. R. The influence of variations in the supply of carbohydrates on the process of nectar secretion. New Phytol. 51 : 294-300. 1952.
21. Zimmermann, Martin. Papierchromatographische Untersuchungen ueber die pflanzliche Zuckersekretion. Berl. Schweiz. Bot. Gesell. 63 : 402-429. 1953.

THE EFFECT OF INFECTIOUS ATROPHIC RHINITIS ON WEIGHT FOR AGE IN SWINE[1]

F. K. KRISTJANSSON AND RONALD GWATKIN[2]

Canada Department of Agriculture

[Received for publication October 5, 1954]

ABSTRACT

Two hundred and thirty-four pigs were examined *post mortem* for evidence of infectious atrophic rhinitis. Twenty-one per cent were found to be infected. Only three of the 27 dams which produced both infected and normal pigs had rhinitis lesions. Rhinitis-infected pigs were found to be significantly lighter at 56, 84, 112, 140 and 168 days of age than their normal litter mates. An analysis of birth weights of infected pigs and their non-infected litter mates indicates that pigs which are born light may be somewhat more susceptible to infection than their heavier litter mates.

For a number of years swine producers in Canada have been concerned over the incidence and effect of infectious atrophic rhinitis in their herds. Up to the present time no adequate control measures for this disease have become available. So far as the authors are aware, there is no published information on extensive studies of the relative gains made by infected and non-infected pigs as determined by post-mortem examination.

During the spring of 1952 post-mortem examinations revealed that there was considerable rhinitis among pigs raised at the Central Experimental Farm, Ottawa. Plans were made to conduct a rhinitis survey on the complete herd in the fall of 1952, and, from the routine records taken in connection with the swine breeding work, to determine the effect of rhinitis upon weight for age.

MATERIALS AND METHODS

All pigs born in the fall of 1952, which survived past four weeks of age were subjected to post-mortem examination. Some of these pigs died or were destroyed prior to reaching market weight. Two hundred and thirty-four pigs were examined at the Animal Diseases Research Institute. Of these pigs, 84 which did not have rhinitis lesions had no rhinitis-infected litter mates, and 8 died prior to 8 weeks of age. The remaining 142 pigs surviving beyond 8 weeks of age had come from litters that contained both infected and normal pigs. The records for this group provided the material for the study of the effect of rhinitis on weight for age. Twenty-seven of the 37 sows which farrowed were subsequently examined *post mortem* for rhinitis lesions.

All pigs were weaned at 56 days of age and self-fed in litter groups from that time on. A.R. ration No. 1[3] was fed for the period from 56 to 112 days of age and A.R. ration No. 2[3] from 112 days of age to 200 lb. live weight. Pigs were weighed at birth, 56, 84, 112, 140, and 168 days of age.

[1] Contribution from Animal Husbandry Division, Experimental Farms Service, Ottawa, Ont.; and Animal Diseases Research Institute, Production Service, Hull, Que.

[2] Animal Geneticist and Senior Research Officer, respectively.

[3] Described in "National Bacon Hog Policy", issued by Production Service, Canada Department of Agriculture. 1952.

The differences in weight between infected and normal litter mates at each of the above ages were tested for significance by means of analyses of variance for hierarchial classifications, as outlined by Snedecor (3). The differences between diagnoses within litters adjusted for differences in birth weight were tested by means of covariance analyses. Facilities did not permit individual feeding, and for this reason no data on feed efficiency for infected and normal pigs are available.

RESULTS AND DISCUSSION

Of the 234 pigs examined, 49 had rhinitis and 185 were normal. Infected pigs were found in 22 out of 37 litters examined. A summary of the incidence of infectious atrophic rhinitis lesions in pigs and their dams is given in Table 1.

TABLE 1.—SUMMARY OF INCIDENCE OF RHINITIS LESIONS IN PIGS AND THEIR DAMS

	Number of dams	Number of dams with infected litters	Number of pigs with rhinitis lesions	Number of pigs with no rhinitis lesions
Dams with rhinitis lesions	3	3	10	15
Dams with no rhinitis lesions	24	16	34	115

It is known that sows with active nasal lesions can infect their pigs. It is also possible that some of the dams which did not show lesions at necropsy may have been carriers of infection and may have infected their litters. The existence of this possibility has been demonstrated by Gwatkin *et al.** in the case of one of four sows examined in the course of previous experimental work. It has also been demonstrated (1) that artifically infected pigs can infect litter mates left in contact with them, and that rhinitis produced with pure cultures of *Pasteurella multocida* also can be transmitted by contact (2).

Thus the infection pattern shown in Table 1 does not exclude the possibility that (*a*) sows which did not display nasal changes at necropsy may, nevertheless, have carried the infective agent or agents, or that (*b*) infection may have spread from other pens.

The average weights of infected pigs and their normal litter mates are presented in Table 2.

In this study infected pigs were found to be significantly lighter at birth than their litter mates which did not contract rhinitis. The most reasonable conclusion is that the pigs with lighter birth weights are more susceptible to infection than are their heavier litter mates.

The differences between infected and normal litter mates, as indicated in the first section of Table 2, are all highly significant. In order to determine if these differences were diagnoses differences, and not primarily due to the lighter birth weights of infected pigs, covariance analyses were performed

* Gwatkin, R., and Dzenis, L. Rhinitis of swine: Effect of streptomycin in the prevention of natural infection in pigs. Prog. Rept. 21. Anim. Path. Division, Canada Dept. of Agriculture, March 6, 1953. (*Unpublished*).

TABLE 2.—AVERAGE WEIGHTS (LB.) OF RHINITIS PIGS AND THEIR NORMAL LITTER MATES

	Uncorrected for birth weight					Corrected for difference in birth weight		
Age	Number	Rhinitis	Number	Normal	Disadv. of rhinitis pigs	Rhinitis	Normal	Disadv. of rhinitis pigs
Birth	45	2.7	97	2.9	0.2**	—	—	—
56 days	45	24	97	33	9 **	25	33	8**
84 days	28	47	77	58	11 **	49	57	8**
112 days	26	83	67	98	15 **	85	97	12**
140 days	26	121	67	140	19 **	123	139	16**
168 days	25	167	65	187	20 **	170	186	16**

** All differences are significant ($p = < 0.01$).

to test the significance of differences between diagnoses means adjusted for birth weight. As will be seen in the latter part of Table 2, the infected pigs are still significantly lighter than their normal litter mates. It is concluded that rhinitis decreases growth rate.

The effect of rhinitis on weight for age as shown by this study is important from an economic standpoint, and also as a possible source of variation in swine experiments. Rigorous randomization of pigs to treatments will reduce the possibility of error from this source of variation to a minimum. At the same time, it is desirable that experimental pigs should be examined *post mortem* as a routine measure in order to determine the incidence of lesions of rhinitis.

REFERENCES

1. Gwatkin, R., P. J. G. Plummer, J. L. Byrne, and R. V. L. Walker. Rhinitis of swine. III. Transmission to baby pigs. Can. J. Comp. Med. and Vet. Sci. 13 (1) : 15-28. 1949.
2. Gwatkin, R., L. Dzenis, A. S. Greig, and C. Grinewitsch. Rhinitis of swine. IX. Further studies on aetiological agents. Can. J. Comp. Med. and Vet. Sci. *In press*.
3. Snedecor, G. W. Statistical methods. 4th ed. The Iowa State College Press, Ames, Iowa. 1946.

FLORISTIC CHANGES FOLLOWING THE CUTTING AND BURNING OF A WOODLOT FOR BLUEBERRY PRODUCTION[1]

I. V. HALL[2]

Plant Pathology Laboratory, Kentville, N.S.

[Received for publication December 13, 1954]

ABSTRACT

This paper traces changes over a period of 4 years in the plant population after a woodlot in southwestern New Brunswick was cleared of trees and burnt over annually for production of blueberries (*Vaccinium angustifolium* and *V. myrtilloides*). The expected increase in blueberry plants did not occur. Instead, the area was rapidly occupied by *Dennstaedtia punctilobula* (hay-scented fern), by vegetative spread of sparse and repressed individuals of the woodland, and by *Carex communis*, *Rumex acetosella*, *Rubus idaeus* var. *strigosus*, and other plants which probably developed from seeds. Characteristic woodland herbs such as *Lycopodium annotinum*, *L. obscurum*, *Maianthemum canadense*, and *Coptis groenlandica*, disappeared after two years of treatment.

Clear-cutting and burning within a period of a year is too drastic a treatment for survival and development of repressed plants of *Vaccinium* usually present in woodland adjoining blueberry fields. Results of present local practice led to the recommendation that the woods be opened gradually or cleared in narrow marginal swaths to allow invasion by vigorous blueberry rhizomes.

INTRODUCTION

In the Atlantic provinces of Canada many pastures, hayfields, and woodlots which had become agriculturally unproductive have now been converted to the production of blueberries of the native "lowbush" type (*V. angustifolium*[3] the lowbush blueberry long known as *V. pensylvanicum* var. *angustifolium* and *V. myrtilloides* the sour-top blueberry also known as *V. canadense*). The deterioration of the old permanent pastures and, to some extent, the hay fields, has been due in a great degree to the gradual encroachment of the blueberry bushes which the climate and soils of the area seem to favour. Selective grazing by livestock (4) tends to weaken the palatable grasses and clovers and allows the persistence of the less palatable ericaceous shrubs. In Charlotte County, New Brunswick, blueberry fields so developed range in size from one-half to 200 acres. Because of better air drainage and less danger of damage to flower buds by late spring frosts, these fields are located on the summits and upper slopes of low ridges rather than in the valleys.

The improvement of these wild blueberry stands to the status of commercially productive fields has been accomplished by a planned program of burning every 3 years. This burning effectively prunes the blueberry bushes by killing the old stems to within a half-inch of the surface of the

[1] Contribution No. 1412, from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Canada. Based on part of a thesis submitted to the Graduate School at Cornell University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] Assistant Botanist.

[3] Scientific nomenclature follows Gray's "Manual of Botany", 8th ed., by M. L. Fernald.

ground and induces the development of new growth on which fruit is produced uniformly and in greater abundance. In commercial practice, the fields are covered with straw in the fall and burnt over early in the spring. During the summer following the spring burn vegetative growth occurs in the form of vigorous sprouts from buds of the rhizome or latent buds of the remnant stem. Flowers and fruits appear on the stems in subsequent years but after 2 years the crop of berries tends to decline, so the fields are reburnt.

There is general knowledge among those who gather blueberries that they are found in greater abundance after a fire has burned over a portion of woodland. The origin of the bushes has always been somewhat of a mystery and several explanations have been put forward. It has been thought that seeds were carried in by birds and found an open bed for establishment on the denuded soil, or dormant seeds already in the forest soil have been stimulated to germination by the minerals released from the ash of the burnt trees. It was thought that woodland, particularly within a blueberry area, could be converted quickly into a commercial blueberry field by a planned process of burning. Ecological studies were commenced in 1949 to determine if this were so and to study the plant succession which took place and its relation to environmental factors such as burning, weather, light and soil.

LITERATURE REVIEW

The importance of fire as an ecological factor has been described by various authors during the past 10 years. In general, coniferous trees and most of the understory species are killed outright, but plants which have parts far enough below ground level to escape burning, and those which can sucker, survive and grow readily in the subsequent years. These plants then become the dominant elements of the population. In the New Jersey pine region, Buell and Cantlon (2), studying the effects of controlled burning, found that in the most frequently burned plots *Vaccinium vacillans* became relatively more abundant in the undergrowth of pines. In old blueberry fields in Quebec, Belzille (1), found such plants as sheep laurel, sweet-fern, and various other shrubs and ferns with underground root systems to be favoured as much as the blueberry by burning. Camp (3) reports that changes in proportions of the various *Vaccinium* species making up the population of Maine blueberry fields occurred as the result of repeated burning. Martin (5) has described the various changes in the vegetation of communities 1, 2, 6, 9, 10, 22, 29 and 40 years after burning by forest fires. On all sites he found that all blueberry plants arose from pre-existing rhizomes. The dominant vegetation immediately after fire on well drained sites was *Pteridium aquilinum*, *Cornus canadensis*, *Kalmia angustifolia* and *Vaccinium angustifolium*. He found that "The increase of a heath plant cover on a burned-over area usually indicates severe forest treatment over a long period of time on the poorer soils of the region". Heath plants were shaded out by developing forest trees but they persisted for many years.

MATERIALS

Location

The present investigation was carried out at the Blueberry Experimental Substation, operated by the Canada Department of Agriculture, and located at Tower Hill in southwestern New Brunswick (45° 18′ N. and 67° 17′ W.), within the influence of the climate associated with the Bay of Fundy. The experimental area is located on a slope near the top of one of the larger ridges in the area at an elevation of 500 feet. In the early days of settlement this land was cleared and farmed, but, like most other farms in the region, it is now unprofitable to operate. Natural invasion of blueberry had taken place and in 1949 it was turned over to blueberry production. The woods selected for study bordered on one of the fields well occupied by blueberry plants.

Nature of Woods

The woodland consisted of a second-growth stand of medium-sized trees, apparently of even age and 25 years old, and characteristic of much of the woodland of the area. *Abies balsamea* (balsam fir) and *Picea rubens* (red spruce) predominated with scattered individuals of *Betula populifolia* (wire birch) and *B. lutea* (yellow birch), *Acer saccharum* (sugar maple), *Populus tremuloides* (aspen and other deciduous trees).

Originally the forest on the site was beech, sugar maple, yellow birch, red spruce, hemlock and white pine (6), but after a long and varied history at the hands of man, the original conditions and composition had become quite changed. The last general clearing or thinning was apparently not accompanied by fire, and the land must have been populated at first with a thick stand of wire birch, aspens and red maple under which the fir and spruce established themselves and eventually became dominant. In some places these conifers came up so thickly that some of their members were suppressed to slender, weak individuals, only a few feet high, by the shade of the few which were successful and had gained a height of 20 or 30 feet and 6-8 inches in diameter. The litter under such trees consisted of a deep mantle of blackened needles and twigs on which herbaceous species were completely absent. Elsewhere, wherever the trees were more widely spaced, or where the canopy was controlled by deciduous species, the leaf-litter and floor-species were correspondingly different. The characteristic floor species, when present, were *Maianthemum canadense*, *Cornus canadensis*, *Coptis groenlandica*, *Lycopodium obscurum*, *L. annotinum*, *Trientalis borealis*, *Vaccinium myrtilloides*, *Aralia nudicaulis*, *Dennstaedtia punctilobula* and seedlings of *Acer rubrum* and *Abies balsamea*. All, except the seedlings which did not survive more than a year or two, are species which tended to form colonies from underground propagating organs, and these colonies were distributed without noticeable relationship to the canopy-species. The density and vigour of individuals, however, depended on the openness of the canopy but in no place was this sufficient to allow them to reach fullest vigour. The *Vaccinium* and *Aralia*, for example, are noticeably depauperate and not flowering or fruiting; the *Dennstaedtia* occur as small spaced fronds, and even the *Lycopodium* species seldom form strobili.

Soil

The soil was a typical acid podzol, a gravelly silt loam with larger fragments of the local shale. The very black appearance of the 3-inch layer of organic matter was due to the partly decomposed plant debris. A grey leached layer of A_2 existed at a depth of from 3 to 5 inches. Extending from 5 inches downward was a chocolate brown B_2 layer which in some places reached a depth of 18 inches. Very often the B horizon rested on bedrock. Its lower limits in some cases showed mottling which indicated poor drainage. Where the soil was deeper, the C horizon consisted of glacial till and also had a grey mottled appearance.

Soil analyses on the organic matter layer from both the woods and burned plot were made by the Chemistry Laboratory, Science Service Laboratory, Kentville, N.S. The soil from the burned plot was very high in all elements, including nitrate nitrogen, and the fertility of the burned area was much higher than that of the woods.

Weather

Rainfall, which amounts to an average of 16 inches during the growing season of May to September, contributes the main portion of the total soil moisture. On the sloping and irregular land, however, the distribution of soil water is modified greatly by seepage and the shallowness of the bed rock. Plant growth is influenced by frequent fogs which sweep in from the coast. In the summer period of 1950, for example, there were 47 foggy days—an unusually large number. During the latter part of May plants may be injured by frost, as the frost-free period extends from approximately June 1 to September 15.

Light

Under the heavy forest canopy much of the red and blue light is filtered out, leaving the yellow and green for photosynthesis. Shirley (7) found that more important than light quality is the reduction in intensity. In this case it was from full sunlight to less than 1 per cent under the spruce and fir (Table 3). To date no satisfactory method of determining total solar radiation has been found.

METHODS

The experimental area was marked out in the woods in June 1949 and cleared of trees that fall. The area was not burned until April 1951. Burning was also practised in 1952. Suckers, arising from the stumps of former trees and shrubs, were pruned in the fall previous to burning.

Floristic changes were recorded on sampling plots within the area in early June of each year. A belt transect, 43 yards long by 1 yard wide and permanently marked, sampled much of the variation present in the woodland. It was subdivided at yard intervals for recording purposes. Each plant, or upright stem of a clonal plant, was counted and its position recorded on a chart. Also a 10×10 yd. plot, marked off adjacent to the transect quadrat, was sampled by a quadrat frame of 0.04 sq. m., thrown one hundred times within it. At each position the species were listed and the amount of their foliage cover, estimated to the nearest tenth of the frame area, was recorded. Bare ground was considered a unit. These readings were converted respectively to frequency index and percentage of ground-cover.

TABLE 1.—NUMBER OF STEMS OF EACH SPECIES PRESENT ON THE BELT TRANSECT IN JUNE FOR THE YEARS 1949 TO 1952

Species	1949	1950	1951	1952
A. Species which declined and after burning a year later completely or almost completely disappeared		Woodlot clear-cut, Oct. 1949	Area burnt over, April, 1951	Strawed and burnt over, April, 1952
Lycopodium annotinum	151	33	0	0
Lycopodium clavatum	1	0	0	0
Lycopodium obscurum	266	84	3	0
Lycopodium complanatum	5	0	0	0
Dryopteris phegopteris	2	1	0	0
Abies balsamea	43	17	0	0
Smilacina racemosa	1	0	0	0
Maianthemum canadense	389	180	67	24
Coptis groenlandica	136	0	1	1
Pyrus melanocarpa	1	0	0	0
Pyrus americana	1	0	0	0
Pyrola rotundifolia	12	1	2	0
Viburnum cassinoides	5	0	0	0
B. Species which were irradicated by bush cutting in the fall of 1950 and 1951				
Betula populifolia	0	193	174	11
Fagus grandifolia	0	0	13	0
Amelanchier laevis	0	2	3	0
Acer rubrum	8	7	5	0
C. Species which increased after treatment				
Dennstaedtia punctilobula	144	326	2326	6359
Pteridium aquilinum	0	0	11	7
Danthonia spicata	0	0	0	28
Panicum lanuginosum	0	0	0	1
Carex communis	9	2	103	121
Rumex acetosella	0	0	18	8
Spiraea latifolia	3	0	1	3
Rubus idaeus var. strigosus	0	0	34	180
Prunus pensylvanica	0	0	0	263
Acer saccharum	0	0	0	12
Viola cucullata	1	0	0	3
Aralia hispida	0	0	0	1
Trientalis borealis	24	1	0	17
Sambucus pubens	0	0	0	1
Solidago rugosa	0	0	27	0
Aster lateriflorus	0	0	0	33
Chrysanthemum leucanthemum	0	0	0	1
Lactuca biennis	0	0	14	1
Hieracium pilosella	0	0	0	3
Hieracium aurantiacum	0	0	0	4
Hieracium pratense	0	0	7	0
D. Species which remained relatively constant				
Osmunda claytoniana	2	2	4	6
Aralia nudicaulis	14	9	15	9
Cornus canadensis	217	121	406	291
Vaccinium myrtilloides	45	44	71	77
Vaccinium angustifolium	3	2	0	3

RESULTS AND DISCUSSION

Following the cutting of the trees and burning of slash (Figure 1) a striking change took place in the nature and composition of the vegetation of the area.

A complete list of the species present on the 43 square yards of the belt transect in each year is given in Table 1. In 1949 the woodland was still intact and only the floor species are recorded in this column.

In Table 2 are figures obtained by sampling the square plot by random quadrats adjacent to the transect. In general the results are similar, although there is a somewhat smaller number of species.

Species listed in Section A of Tables 1 and 2 are all native species characteristic of woodland and were evidently susceptible to the desiccation resulting from removal of the forest canopy or were destroyed by the burning of the duff layer containing their runners. For example, *Lycopodium obscurum* with rhizomes 2-3 inches below the ground level persisted longer than *L. annotinum* with a surface rhizome. Removal, alone, of the forest canopy has been sufficient to destroy *L. clavatum* and *L. complanatum*.

During the years 1950 and 1951 there was extensive sucker growth from the root crowns of *Betula populifolia*, *Fagus grandifolia*, *Amelanchier laevis* and *Acer rubrum*. In the usual fall pruning for weed control in blueberry fields, these suckers were cut as close to the surface of the ground as possible. By 1952 (see Section B of Tables 1 and 2) no sucker growth developed, suggesting that no food was left in the roots.

Section C of Tables 1 and 2 contains the species characteristic of clearings, or introduced weeds common in the area. Some of them such as *Dennstaedtia*, *Pteridium* and *Trientalis* were present in the original woods and escaped the burning by virtue of their deep runners. These organs were respectively 2, 4 and 1 inches below the ground. With increased nutrients, full sunlight and no competition after clearing and burning, plants of the hay-scented fern grew rapidly. The fronds as well as the underground rhizomes grow throughout the entire growing season.

The following plants made their appearance and presumably arose from seeds already in the soil, or brought in with the straw used in burning or by the wind. Species growing from seeds in the soil or hay were *Viola cucullata*, *Spiraea latifolia* and *Carex communis;* from seeds in the hay were *Danthonia spicata*, *Agrostis tenuis*, *Panicum lanuginosum* var. *lindheimeri*, *Rumex acetosella*, *Chrysanthemum leucanthemum* var. *pinnatifidum*; from seeds carried by the wind *Acer saccharum;* from both wind and hay *Solidago rugosa*, *Aster lateriflorus*, *Erigeron strigosus*, *Lactuca biennis*, *Hieracium pilosella*, *H. aurantiacum* and *H. pratense*. A number of fleshy-fruited species have probably been introduced by birds. These include *Amelanchier laevis*, *Rubus idaeus* var. *strigosus*, *Prunus pensylvanica*, *Aralia hispida* and *Sambucus pubens*.

Certain species found in Section D of Tables 1 and 2 did not change greatly in number of individuals. The survival of *Cornus canadensis*, *Aralia nudicaulis* and *Osmunda claytoniana* is attributed to the fact that their rhizomes are from 2 to 3 inches below the ground level, hence, deep

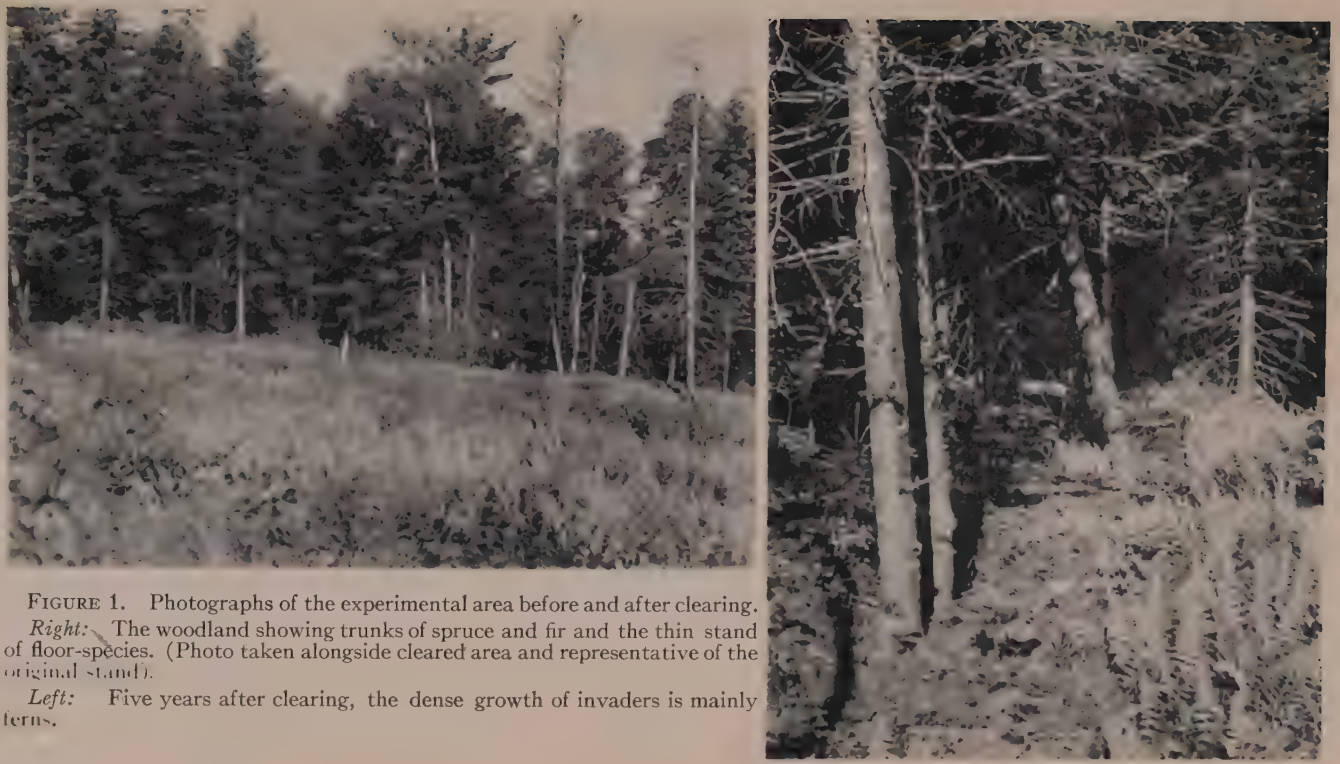

FIGURE 1. Photographs of the experimental area before and after clearing.

Right: The woodland showing trunks of spruce and fir and the thin stand of floor-species. (Photo taken alongside cleared area and representative of the original stand).

Left: Five years after clearing, the dense growth of invaders is mainly ferns.

enough to escape killing by fire. Similarly, adapted species with rhizomes from 0-4 inches are the blueberries. Within most woods in the vicinity a few very weak plants of lowbush blueberry and sour-top can generally be found on careful search. These have only two or three small leaves and give the appearance of seedlings; but they are actually the surviving tops of very old plants which possibly date from the time woods were cleared. They never flower or fruit under light of low intensity.

A number of habitats were examined to assess the effect of light on blueberry growth. Where shade was heavy, blueberry growth was poor; nearby, in an area where trees had fallen or been cut, vegetative growth was vigorous and in open areas blueberry plants were found to flower and fruit. As a result of these observations, recordings were made under different canopies with a General Electric light-meter. Table 3 presents a summary of these observations. Since light intensities vary with weather conditions and time of day, it was considered best to express data in terms of percentage of full sunlight rather than foot-candles.

Although surviving as meagre, depauperate stems, blueberry plants growing in thick woods will develop into stronger plants when given more light. They, and not seedlings, are undoubtedly the source that should be counted on for establishing new blueberry stands after clearing densely covered woodland.

To determine the prevalence of blueberry seed in soil from dense woodland three samples of soil were dried and screened for seed analysis. The samples were cut to a depth of 3 inches and covered an area of 1 square foot. After being dried, the samples were sifted through a 20-mesh sieve through which blueberry seeds will pass and then on a 50-mesh sieve which retains the seed. A close examination of this retained material revealed the presence of 8 blueberry seeds in one sample but none in the other two. Among the other seeds found were those of *Betula populifolia* to the extent of over 100 seeds per sample.

STATISTICAL ANALYSES OF DATA

An analysis of variance has been applied to the figures from individual square yard sections to determine if the differences in amounts of certain species were significant between years and sections. With one exception, *Dennstaedtia punctilobula* which was uniformly distributed, there was a significant difference between sections for all species tested. This shows the great diversity of the plant population.

Dennstaedtia punctilobula showed a highly significant increase each year. *Carex communis* also showed a highly significant increase of individuals with years. *Cornus canadensis*, although spreading as a weed in certain blueberry fields, showed no significant increase in the 4 years on the experimental area. The decrease of *Maianthemum canadense* was significant.

Although the number of plants of the sour-top blueberry increased from 45 to 77 on the transect in 4 years, the gain was not statistically significant. In 1952 the three same sections as in 1949 had 1 plant each of lowbush blueberry. No new plants had arisen.

In the square plot the unit "bare ground" showed a statistical decrease between years, and confirmed the increase in plant cover.

TABLE 2.—FREQUENCY OF OCCURRENCE AND PERCENTAGE OF FOLIAGE COVER OF SPECIES IN 100 RANDOM SAMPLES WITHIN THE 10 × 10 YARD PLOT

Species	Frequency index				Percentage foliage cover			
	1949	1950	1951	1952	1949	1950	1951	1952
A. Species which declined and after burning a year later completely or almost completely disappeared								
Bare space*	16	32	14	2	81.2	85.4	43.4	18.2
Mosses	30	6	3	0	6.0	0.0	0.8	0.0
Lycopodium annotinum	32	16	0	0	4.1	1.7	0.0	0.0
Lycopodium clavatum	2	0	0	0	0.0	0.0	0.0	0.0
Lycopodium obscurum	27	18	2	0	2.5	2.3	0.7	0.0
Lycopodium complanatum	1	0	0	0	0.0	0.0	0.0	0.0
Abies balsamea	11	4	0	0	0.0	0.0	0.0	0.0
Uvularia sessilifolia	1	1	0	0	0.0	0.0	0.0	0.0
Maianthemum canadense	42	17	0	1	3.0	0.0	0.0	0.0
Habenaria psycodes	1	0	0	0	0.0	0.0	0.0	0.0
Coptis groenlandica	10	0	0	0	0.0	0.0	0.0	0.0
Pyrus melanocarpa	0	1	0	0	0.0	0.0	0.0	0.0
B. Species which were eradicated by bush cutting in the fall of 1950 and 1951								
Betula populifolia	0	8	7	0	0.0	6.7	7.5	0.1
Amelanchier laevis	0	1	1	0	0.0	0.0	0.0	0.0
Acer rubrum	4	0	1	0	0.0	0.0	0.0	0.0

C. Species which increased after treatment								
Dennstaedtia punctilobula	21	6	41	45	1.6	3.2	23.0	35.6
Pteridium aquilinum	0	1	6	7	0.0	0.2	4.0	4.0
Danthonia spicata	0	0	19	9	0.0	0.0	5.0	2.3
Agrostis tenuis	0	0	1	0	0.0	0.0	0.0	0.0
Panicum lanuginosum	0	0	0	1	0.0	0.0	0.0	0.0
Carex communis	3	1	21	26	0.0	0.0	7.5	5.6
Rumex acetosella	0	0	11	48	0.0	0.0	3.0	21.6
Rubus idaeus var. strigosus	0	0	7	37	0.0	0.0	2.5	8.2
Prunus pensylvanica	0	0	11	7	0.0	0.0	0.8	0.6
Acer saccharum	0	0	0	0	0.0	0.1	0.1	0.0
Viola cucullata	0	0	0	1	0.0	0.0	0.0	0.1
Aralia hispida	0	0	0	4	0.0	0.0	0.0	1.1
Trientalis borealis	5	0	3	2	0.0	0.0	0.0	0.1
Erigeron strigosus	0	0	1	0	0.0	0.0	0.0	0.0
D. Species which remained relatively constant								
Osmunda claytoniana	1	1	2	2	0.0	0.0	0.2	0.8
Aralia nudicaulis	2	1	4	1	0.0	0.1	0.2	0.2
Cornus canadensis	12	6	9	5	0.0	0.1	0.8	1.4
Vaccinium myrtilloides	8	9	7	2	1.6	0.2	0.5	0.1

* Under "Frequency Index", the figures for Bare Space indicate number of samples completely without plants, and under "Percentage Foliage Cover", the proportion not covered by plant foliage.

TABLE 3.—LIGHT INTENSITIES FOUND UNDER VARIOUS CANOPIES RESULTING IN DIFFERENT STAGES OF GROWTH

Threshold	Percentage of full sunlight					
	Vacc.	Dennst.	Lycop.	Cornus	Coptis	Maianth.
For minimum growth (i.e. just survival) spruce-fir canopy	0.5	1.0	0.5	1.0	0.5	2.5
For moderate vegetative growth (but not flowering) wire birch canopy	10.0	10.0	15.0	15.0	20.0	10.0
For flowering and fruiting openings in forest canopy	50.0	50.0	35.0	50.0	35.0	35.0

APPLICATION OF FINDINGS

This study shows that on this site clean-cutting of forest trees when followed in one year by burning failed to give a good stand of blueberries. No blueberry seedlings made their appearance. After growing in heavy shade, many of the small blueberry plants will be killed by a subsequent fire. If, however, the burning is delayed several years until vigorous plants develop greater success will probably be obtained. In such cases weed control becomes a very important factor. If there are weak blueberry plants in the ground cover, the forest trees should be selectively cut until enough light enters to encourage vigorous plant development before the plants are subjected to further treatment. Where areas have been thinned by pulpwood cutting, vigorous plants have been found in the woods. These plants when given a favourable environment will act as centres from which new rhizomes will spread.

When it is desired to enlarge a producing field by clearing land from adjoining woodland, blueberry growers at Tower Hill have found it best to cut and burn a narrow strip, 2 or 3 feet wide, each year. This procedure stimulates the spread of existing plants rather than the growth of seedlings.

REFERENCES

1. Belzille, A. The problem of weeds in blueberry barrens. Proc., 5th meeting East. Sect. Nat. Weed Comm., 1951, pp. 120-124. 1952.
2. Buell, M. F., and J. Cantlon, Effects of prescribed burning on ground cover in the New Jersey pine region. Ecology 34 : 520-528. 1953.
3. Camp, W. H. The North American blueberries with notes on other groups of *Vacciniaceae*. Brittonia. 5 (3) : 203-275. 1945.
4. Hall, I. V. Weed control by grazing. Progress Report, Blueberry Substation, Tower Hill, N.B. (*In press*).
5. Martin, J. L. The vegetational characteristics of certain forest burn communities on the southern upland of Nova Scotia, with observations on secondary succession. M.Sc. thesis, Dalhousie University, Halifax, N.S. 1954.
6. Nichols, G. E. The hemlock-white pine-northern hardwood region of eastern North America. Ecology 16 : 403-422. 1935.
7. Shirley, H. L. The influence of light intensity and light quality upon the growth of plants. Amer. J. of Bot. 16 : 354-390. 1929.

PARAFFIN AND MICROCRYSTALLINE WAXES AS CHEESE COATING MATERIALS[1]

O. R. Irvine,[2] D. H. Bullock[2], and R. E. Edmond[3]

[Received for publication November 16, 1954]

ABSTRACT

Petroleum waxes of varying physical properties were examined as cheese coating materials. Test slices of Cheddar cheese, after immersion in hot wax, were examined for thickness of wax film, weight loss over a 30-day period, resistance to impact damage and control of mould growth. Two batch-produced waxes and one of the semi-commercially prepared waxes gave superior protection to cheese as compared to refined paraffin. However, weight losses were slightly lower where commercial cheese waxes were used.

The effects of varying times and temperatures of application upon the protection afforded to cheese by two types of wax were determined. Blends of paraffin and microcrystalline wax, as well as waxes containing admixtures of mineral oil, were also studied. Some waxes imparted undesirable flavour to cheese.

Three groups of triplet-size Cheddar cheese were given different wax treatments, and shrinkages determined after 11 months' storage. Double-coated cheese lost significantly less weight and rinds remained in excellent condition.

INTRODUCTION

Methods of protecting the surfaces of cheese during storage have long been a problem in this industry from the standpoint of reducing waste. A suitable protective coating should: (*a*) retard loss of moisture from the surface of the cheese; (*b*) provide protection from mould growth and other destructive agents. The problem is particularly serious in Cheddar cheese, which are usually cured for long intervals at temperatures favourable to the growth of surface moulds.

Losses which occur during storage as a result of rind-rot, cracked rinds, and shrinkage (loss in weight), as well as damage from mould and mites, are appreciable. A recent estimate shows that, in 1952-53, 15 per cent of the cheese originally graded as "Canada First Grade" was lowered in grade after storage, principally because of defective rind development (2). It is felt that the correct application of better waxes could assist in reducing these losses.

Paraffin wax from the petroleum refining industry has been used for many years to coat cheese. The early recommendations of Doane (5) on methods of applying paraffin wax to cheese still serve as a basis for using this product. Where proper care in applying wax is taken, it serves as an economical, easily applied and a fairly longlasting protective coating.

Paraffin wax has some shortcomings, however. Its tendency to crack or shatter on impact at low temperatures is a serious fault. Associated with this is a tendency for such coating materials to become flaky or scaly and in time to lose their protective value. Furthermore, the rate of

[1] Joint contribution from the Department of Dairying, Ontario Agricultural College, Guelph, Ont., and Imperial Oil Research Laboratories, Sarnia, Ont.

[2] Assistant Professor and Lecturer, respectively.

[3] Agricultural Chemist.

moisture evaporation from paraffined surfaces, while greatly retarded, is not entirely prevented, with the result that considerable shrinkage occurs and rinds become objectionably thick.

Some microcrystalline waxes from petroleum have greater flexibility, are better moisture vapour barriers and consequently have been utilized to coat cheese. The sources and properties of these products have been reviewed by Bennett (3) and Warth (14), while their use as cheese waxes has been discussed by Van Slyke and Price (13) and Swett (6). Bennett (3) states that there is no prescribed rule by which the microcrystalline waxes may be distinguished. Their crystal size is smaller and is needle-like in structure. They have higher tensile strengths and melting points, as well as higher penetration values and refractive indices. They bind solvent oils better than paraffin wax and thus prevent the former from being sweated out of blends. The terms "microcrystalline" and "amorphous" are sometimes used synonymously, although the former is more accurate. Formulae containing microcrystalline waxes, as well as fatty acids, synthetic rubber, polyethylene and mould inhibitors, have been patented in the United States (7-12).

Warth (14) has shown that oxidation products, resulting from high temperatures and exposure to certain metals, can induce off-flavours in wax. Exposure to temperatures 100° F. above the melting point of the wax for 24 hours produced measurable decomposition (14). Vessels made of brass, copper, zinc and monel metal are listed as undesirable for contacting melted wax, while aluminium and stainless steel are satisfactory. Antioxidants may be added to delay deterioration.

Equipment and recommended procedures for waxing cheese are given in a recent publication of the Canada Department of Agriculture (1). No specifications are made for the paraffin to be used, except that it should be clean and clear and give a thin, somewhat flexible coating.

This paper gives the results of a study of a number of experimentally produced waxes as to their suitability for coating Cheddar cheese (Table 1). In addition, tests were conducted to learn how variations in methods of applying waxes affected the results. Another part of the project which was conducted concurrently dealt with the use of certain mould-inhibiting wax additives (4).

MATERIALS AND METHODS

Except for four commercially available cheese waxes which were included for comparative purposes, the waxes studied were all experimentally produced materials*. Two refined paraffin waxes from plant production were also studied. In a subsequent series of experiments, seven additional waxes, prepared under semi-commercial refinery conditions, were also tested as well as one additional commercial wax.

Cheese slices weighing approximately 600-800 gm. were prepared by making Cheddar cheese in the Stilton size (approximately 10″ in height × 6″ in diameter), pressing for about 16 hours and cutting each into three pieces. Subsequently each piece was returned to the press for 4-5 hours to form a rind on the freshly cut surfaces. After removal from the press

*Produced as experimental products by the Research Department, Imperial Oil Ltd., Sarnia, Ont.

TABLE 1.—PHYSICAL PROPERTIES AND DESCRIPTION OF WAXES*

Wax Code No.	Melting point (° F.)	Per cent of oil content	Penetration (ASTM-D5-25)	Viscosity at 210° F.	Refractive index	Description and type
273	173	3.3	16	61	1.4440	Hard, brittle, high m.p. crystalline
274	148	2.9	23	64	1.4477	Soft, flexible, conventional m.p. microcrystalline
275	115	4.7	40	67	1.4511	Soft, flexible, low m.p. microcrystalline
276	159	2.4	15	49	1.4394	High m.p., paraffin
277	148	1.3	17	49	1.4381	High m.p., paraffin
278	121	2.5	33	48	1.4421	Low m.p., intermediate type
279	110	1.7	165	37	1.4305	Low m.p., high penetration
280	120	1.2	22	41	1.4305	Low m.p., low penetration
A	150.1	4.8	32	51.5	—	Commercial Cheese Waxes
B	150	6.5	29	48.2	—	
C	151.3	7.2	44	77	—	
D	143.2	8.8	38	62	—	

* Inspection data supplied by Research Department, Imperial Oil Limited, Sarnia, Ont.

FIGURE 1. Slices of cheese arranged to determine rates of shrinkage during 30-day test.

these sections were allowed to dry thoroughly at 60° F. for 5 days before waxing. At this time each section was carefully cut into two pieces, each piece thus having one freshly cut surface at the time when the wax was applied.

All the waxes under test were applied to cheese slices at two temperatures—190° F. and 220° F.—the former temperature having been found suitable for coating microcrystalline or blended waxes while the latter was based on Doane's (5) and Van Slyke and Price's (13) recommendations for paraffin wax. All tests were carried out in duplicate and the results in the following tables are the averages of these duplicates.

Immediately before and after immersion the test slices were weighed and the quantity of wax adhering to the surface obtained by difference. The slices were held by three wire hooks and dipped into the molten wax at an angle of 45 degrees. They were also removed from the wax at this angle and held until the wax had congealed. The waxed slices were then stored for one month at 50° F., at approximately 60 per cent relative humidity. The test pieces were so supported that most of the surface area was exposed and were left undisturbed for the full storage period (Figure 1). The temperature of the room was thermostatically controlled within a range of about $\pm$ 1° F., and the air was circulated by a slow speed fan. In addition to determining the shrinkage of the test slices, the surfaces were carefully examined at the end of the storage period for such imperfections as pin-holes, blisters, mould growth or scaling of the wax.

The resistance of the wax coating to shattering was determined by subjecting the test slices to a dropping ball test, conducted as follows: A cast iron ball, 4 cm. in diameter and weighing 226 grams, was dropped a distance of 12 inches on to the rinded, waxed surface of the slice maintained at 50° F. The fracture pattern which resulted from the impact varied from "imperceptible" to one covering a circular area approximately 4 cm. in diameter.

RESULTS

The slices of cheese used in the shrinkage tests were of sufficiently regular outline to allow their surface areas to be measured and hence to

TABLE 2.—PERFORMANCE OF WAXES AS CHEESE COATING MATERIALS—AVERAGES OF DUPLICATE TRIALS ON TEST SLICES OF CHEDDAR CHEESE FOR 30 DAYS AT 50° F. AND 60 PER CENT R.H.

Wax	Temp. applied	Weight of wax applied (mg./cm.²)	Loss in weight (mg./cm.²)	Impact test*	Remarks—conditions on freshly cut surface
273	190	70	33.1	xxxx	Badly cracked, 75 per cent mould covered, cracked
	220	46	27.2**	xxx	and contracted away from edge.
274	190	54	13.0	N	Two 2 cm. mould colonies.
	220	36	19.0	N	10-12 small colonies.
275	190	30	6.2	N	Some blisters and small colonies.
	220	26	9.5	N	Very good.
276	190	51	32.7	xxxx	Contracted from corners, 60 per cent mould.
	220	34	23.1	xxx	Fractures, mould, 7 bubbles.
277	190	43	18.5	xxxx	Contracted from corners—poor.
	220	24	23.0	xxx	Poor on freshly cut surface.
278	190	32	7.5	N	Very good.
	220	22	18.4	N	6 small colonies.
279	190	15	21.0	xx	Very good.
	220	12	34.0	x	Very good.
280	190	37	15.7	xx	Many 2 cm. bubbles and colonies.
	220	24	14.4	x	Many 2 cm. bubbles and colonies.
133° F.	190	37	9.8	xxxx	2 large mould areas.
	220	24	16.2	xxx	Many 2 cm. bubbles and colonies.
138° F.	190	37	15.8	xxxx	25 per cent moulded.
	220	27	14.7	xxx	10 per cent moulded.
A	190	40	0.9	xxx	Four 1 cm. mould colonies, some blisters.
	220	31	2.9	xx	Three 0.5 cm. colonies, small blisters.
B	190	49	2.3	xxx	Some 2 cm. blisters.
	220	29	3.5	xxx	Some 1 cm. blisters.
C	190	58	1.9	N	3 or 4 small colonies, 10-12 4 cm. blisters.
	220	35	2.7	N	Very good, some pin-holes.
D	190	45	2.5	N	Very good, some pin-holes.
	220	38	9.6**	N	Numerous pin-holes.

* Damage from the dropping ball impact test varied from N, indicating no damage, up to xxxx, in which the surface fracture covered a circle about 4 cm. in diameter.
** Single determination.

calculate the weight of the wax film in milligrams of wax per square centimetre (Table 2). The loss in weight occurring during the 30-day storage period is expressed in the same terms.

At both temperatures (190° and 220° F.) it is apparent that the thickness of the film is related to the melting point and the viscosity of the wax at application temperature (3). In every instance the higher dipping temperature resulted in a thinner protective coating. The loss in weight during the 30-day period is related to film thickness, except in the cases where the film contracted from the corners of the slice and resulted in

abnormal shrinkage. This unusual behaviour was closely associated with poor performance on the impact test and hence rules out the use of waxes with these characteristics.

The ability to withstand impact was superior where the waxes were applied at the higher temperature (220° F.), thus indicating that thin wax films have higher resistance to fracturing. In general those which resisted fracturing (as indicated by the test) were satisfactory in other respects and especially with regard to retarding shrinkage and controlling mould growth.

The extremely low melting points of samples No. 275 and No. 279 make them unsuitable as cheese waxes even though their other properties were good, particularly in the case of No. 275. Waxes of this type stick to shelving, boxes, etc., and tend to feel greasy. The waxes with the exceptionally high melting points, on the other hand, were equally unsatisfactory, No. 273, 276 and 277 all being in this category. These wax films fractured easily and often contracted from the corners of the test slices, thus producing high shrinkage values.

Samples No. 274, 278 and 280 exhibited good performance from the standpoint of loss in weight and fracture resistance. The first is preferred because of its higher melting point, low oil content and physical properties similar to those of the commercial waxes studied.

EFFECT OF TEMPERATURE OF APPLYING WAX

The effect of temperature of application (ranging from 160° to 280° F.) on the protective properties of two of the waxes (No. 280 and 133°-136° F. M.P. paraffin) was studied in the same manner as in the tests described above. Results are shown in Table 3. The higher application temperature resulted in a lower weight of wax being applied which in turn generally increased the shrinkage. In other respects, however, the performance of the wax improved with the higher application temperatures. This is reflected in a better resistance to impact damage, fewer and smaller blisters, and greater freedom from mould. These results suggest that, if oxidation of the wax could be controlled, waxing temperatures might be raised in order to control mould growth by heat sterilization of the surface of the cheese.

TABLE 3.—EFFECT OF TEMPERATURE OF APPLICATION UPON PROTECTIVE PROPERTIES
(Dipping time 6 seconds)

Wax	Temp. applied	Weight of wax applied (mg./cm.²)	Loss in weight (mg./cm.²)	Impact test	Remarks—conditions on freshly cut surface
280	160	40	10.8	xxx	2-3 cm. blisters.
	190	28	15.7	xx	Many 2-cm. blisters.
	220	23.5	14.4	x	Many 2-cm. blisters.
	250	17.3	29.6	x	Good.
	280	13.5	33.7	N	Very good.
133° F.	160	42	10.2	xxxx	30 per cent of surface moulded.
	190	37	9.8	xxxx	2 moulded areas.
	220	24	16.2	xxx	Many 2-cm. blisters and some colonies.
	250	23	43.6	xxx	Fairly good.
	280	17	26.2	xxx	Fairly good.

TABLE 4.—EFFECT OF IMMERSION TIME UPON PROTECTIVE PROPERTIES

(Coating temperature 220° F.)

Wax	Immersion time	Weight of wax applied (mg./cm.²)	Loss in weight (mg./cm.²)	Impact test	Remarks—fresh surface
280	2 sec.	21	3.8	xx	Good.
	6 sec.	22.7	5.9	xx	Good.
	18 sec.	14.3	19.4	x	Extremely thin coating in centre of slice.
133° F.	2 sec.	17.8	8.4	xxxx	7- 8 colonies and blisters.
	6 sec.	20.7	5.7	xxxx	7- 8 colonies and blisters.
	18 sec.	12.8	10.4	xxxx	10-12 small colonies; blisters.

EFFECT OF LENGTH OF IMMERSION TIME

The same two samples were used as coatings to study the effect of immersion time (2, 6, 18 seconds) in the wax heated to 220° F. The results of the experiment are shown in Table 4. Increasing the immersion time to 18 seconds resulted in a thinner coating on the cheese and hence a greater loss by evaporation. It is apparent that retaining the cheese in the bath for the 18 seconds resulted in more wax draining from the slice after its removal from the bath. This maximum holding interval did not prevent mould growth on these slices.

The possibility that the hot paraffin might extract moisture from the slice of cheese was considered, but it was found that within the limits of experimental accuracy the difference in weight before and after waxing was equal to the reduction in weight of the wax removed from the bath.

EFFECT OF BLENDING 133°-136° F. M.P. WAX WITH MICROCRYSTALLINE WAXES

Where microcrystalline waxes are used as moisture-vapour barriers it is a common practice to blend them with refined paraffin waxes. Some workers suggest mixing one part of microcrystalline wax with two of 133°-136° F. paraffin. However, Bennett (3) states that the flexible properties of a blend do not increase proportionally with increasing quantities of microcrystalline wax.

Blends of increasing proportions of three microcrystalline waxes and 133°-136° F. paraffin were made, consisting of 20, 40, 60 and 80 per cent of the two commercial and one experimental waxes. The trial was carried out in duplicate as described above. The slices were dipped for 6 seconds in wax at 220° F., and the results are presented in Table 5.

Blends containing larger proportions of flexible waxes resist fracturing better than high proportions of 133°-136° F. paraffin wax. Except for the commercial wax "B" the resistance to fracturing was improved with concentration, in spite of the fact that a thicker film of wax was present.

The tendency exhibited by waxes "B" and "D" to show pin-holes in the films is probably the result of either a higher surface tension or higher viscosity than found in wax No. 278 or the 100 per cent 133°-136° F. paraffin wax.

TABLE 5.—EFFECT OF BLENDING 133°-136° F. PARAFFIN WAX WITH INCREMENTS OF MICROCRYSTALLINE WAXES

(Trial period 28 days at 50° F. and 60 per cent R.H.; waxes applied at 220° F. for 6 seconds)

133° F. wax plus	Weight of wax applied (mg./cm.²)	Loss in weight (mg./cm.²)	Impact test	Remarks—fresh surface
None	22.4	18.6	xxxx	Numerous mould colonies in crevices.
20 per cent B	19.6	9.8	xxxx	Numerous mould colonies in crevices.
40 per cent B	22.9	7.8	xxxx	10-12 small colonies.
60 per cent B	24.2	5.2	xxxx	6 small colonies.
80 per cent B	27.7	8.1	xxx	5 small colonies.
100 per cent B	30.9	6.9	xxx	5 small colonies, pin-holes.
20 per cent D	19.8	8.1	xxxx	10 small colonies.
40 per cent D	21.0	6.3	xxx	12-15 small colonies.
60 per cent D	25.0	5.2	xxx	10 small colonies, pin-holes.
80 per cent D	27.0	6.1	N	12-15 small colonies, pin-holes.
100 per cent D	30.8	11.7	N	Very small colonies, many large pin-holes.
20 per cent 278	17.3	11.6	xxx	Numerous small colonies.
40 per cent 278	20.1	12.2	x	15 small colonies.
60 per cent 278	19.7	10.9	N	10-12 small colonies.
80 per cent 278	21.5	11.6	N	12-15 small colonies.
100 per cent 278	22.4	9.3	N	15-20 small colonies.

TABLE 6.—THE EFFECT OF INCREASING QUANTITIES OF MINERAL OIL IN TWO WAXES (APPLIED AT 220° F. FOR 6 SECONDS, STORED 32 DAYS)

Wax	Added oil (per cent)	Weight of wax applied (mg./cm.²)	Loss in weight (mg./cm.²)	Impact test	Remarks—fresh surface
278	0	22.8	10.4	N	Very little mould or blisters.
	5	21.8	12.3	N	3 per cent moulded.
	7.5	21.4	13.3	N	5 per cent moulded, slightly oily feel.
	10	19.6	12.8	N	3 per cent moulded, slightly oily feel.
	15	20.3	18.0	N	3 per cent moulded, oily feel.
133° F.	0	24.8	11.0	xxxx	20-25 per cent moulded.
	2.5	20.2	12.7	xxx	5 per cent moulded, 1 cm. blisters.
	5	19.7	11.4	xxx	3 per cent moulded, 1 cm. blisters.
	7.5	20.1	10.5	xxx	3 per cent moulded, 1 cm. blisters.
	10	20.9	9.0	xxx	3 per cent moulded, 1 cm. blisters.
	15	23.0	10.5	xxx	3 per cent moulded, 1 cm. blisters.

EFFECT OF ADDING MINERAL OIL TO WAXES

In comparing the physical properties it was noted that the commercial waxes all contained more oil than the experimental waxes. It was, therefore, decided to compare the effect of increasing increments of mineral oil in one of the experimental waxes showing microcrystalline properties, with that of 133°-136° F. M.P. paraffin wax treated similarly. S.A.E. No. 20 lubricating oil was selected and blended with the waxes in the proportions shown in Table 6. In neither case did addition of the oil greatly improve the qualities of the wax. At the higher oil content the waxed surfaces had a somewhat oily feel and lacked the clear lustre of the controls. These were not tacky, however, and adhered to the cheese to the same degree as

the control coatings. Apparently the improved performance of the commercial waxes (see Table 2) from the standpoint of "shrinkage" is not related to the higher oil content.

SHRINKAGE TRIALS USING SINGLE- AND DOUBLE-COATED TRIPLET SIZE CHEESE

In a trial simulating commercial storage conditions, one group of 13 triplet size cheese was coated with a refined paraffin; a second group of 13 was coated with No. 278 wax, while a third group of 13 cheese was double-coated. In applying the single coatings, the cheese was dipped for 6 seconds at 220° F. The double coating was applied to the cheese by first immersing for 6 seconds in the 133°-136° F. M.P. wax, followed by 6 seconds in the No. 278 wax. The cheese, which varied in weight from 24 to 46 pounds, had been turned once each day while drying at 50° F. and 60-70 per cent relative humidity for 4-14 days before treatment. The same storage conditions were maintained for the next 3 months, after which the cheese were moved to a 40° F. room. Weighings were made to the nearest half-ounce at the end of 30, 60 and 330 days. The shrinkages which resulted after 60 and 330 days are given in Table 7.

Shrinkage was relatively negligible in all three lots of cheese at the end of 60 days, with the double-dipped series showing the smallest loss. Shrinkage continued to take place after the cheeses were moved to the lower temperature with the same trends being evident. Despite reduced shrinkage of the double coated cheese, this additional operation is not economical when the additional labour costs are considered. However, since the rinds remained free from mould growth, further study may be warranted, particularly for commercial operations.

In appearance and shatter-resistance, the double-dipped cheese were similar to those protected with the No. 278 wax alone. The weight of wax required was approximately twice as much for the second as for the initial coat.

By the end of the 11-month period, the cylindrical surfaces of the cheese dipped in the 133°-136° F. M.P. wax had become scaly. This condition was noted in only one instance among the cheese coated with No. 278 wax and was entirely absent where the cheese were double-dipped.

TABLE 7.—SHRINKAGE IN TRIPLET-SIZE SINGLE- AND DOUBLE-COATED CHEDDAR CHEESE
(60 days at 50° F. and 60-70 per cent R.H., followed by 270 days at 40°-42° F.)

Wax	Average weight of cheese	Average weight of wax applied	Shrinkage at end of—	
			60 days	300 days
	(lb.)	(oz.)	(oz.)	(oz.)
133° F.	34.30	3.69	2.96	10.69
278	33.57	3.81	2.92	10.12*
133° F. + 278	35.04	5.73	2.38	7.66

*8 cheese.

In most instances both end-surfaces of the double-dipped cheese remained free of mould throughout this storage period. On the other hand, frequently one, and sometimes both ends of the single-dipped cheese exhibited numerous small areas of mould growth. In one or two instances rind-rot in its early stages was becoming evident.

FLAVOURS IMPARTED TO CHEESE BY WAXES

(*1*) *Trials Involving Waxes Produced Under Experimental Pilot Plant Conditions*

In the past little attention has been given to the absorption of undesirable flavours from wax, since the rinds of Canadian export-size Cheddar cheese are usually removed prior to use. In the case of wax-coated rindless cheese or retail cuts, it is essential that no off-flavour be picked up from this source.

The flavours from these batch-produced experimental waxes were studied by a flavour scoring panel of four experienced tasters. Pieces of 8-week old Cheddar cheese (1 × 2 × 3 inches) were dipped in the melted waxes for 6 seconds and then stored for approximately 48 hours at 50° F. before being examined. One series was dipped at 190° F., and a second

TABLE 8.—THE EFFECT OF EXPERIMENTALLY PRODUCED WAXES UPON THE FLAVOUR OF MILD CHEDDAR CHEESE—POSSIBLE RANGE WAS FROM 10 (EXCELLENT, NO OFF-FLAVOUR) TO 1 (HIGHLY OBJECTIONABLE)

Wax	Range of flavour scores for waxing at		Average score for waxing at	
	190° F.	220° F.	190° F.	220° F.
273	9–2	8–4	5.25	6.25
274	8–2	10–6	6.25	8.25
275	10–9	10–9	9.50	9.50
276	6–4	8–2	5.00	4.75
277	6–2	10–6	4.25	8.50
278	8–1	5–1	4.75	3.75
279	10–6	10–8	8.50	9.00
280	10–10	10–7	10.00	9.25
133° F.	9–4	9–7	6.75	8.25
138° F.	10–3	9–5	6.00	7.75
A	9–4	10–8	6.50	8.75
B	10–6	9–5	9.00	7.00
C	9–7	10–4	8.00	6.00
D	10–8	10–7	8.75	8.50

Necessary difference between treatment means for two temperatures at 95 per cent confidence level = 2.0.

one at 220° F. Before being scored for flavour, samples were held at room temperature for two hours; the wax coating was then peeled off and slices $\frac{1}{4}$-inch thick were scored. The samples were coded in such a way that the judges knew only the pairs of samples which had been dipped at the two temperatures, but otherwise were not told the treatment. Table 7 presents the ranges of scores as well as the averages.

It will be noted that the range of scores is quite wide and that in some cases the average is greatly influenced by one diverse score. (In a later panel, this variability was reduced by the panel agreeing on a standard flavour score for one sample before evaluating additional samples). Statistical analyses of the data indicate that the difference between mean scores were in many cases highly significant, the absorbed flavours being attributed to the various waxes. With waxes No. 273, 276, 277 and 278, definite off-flavours such as kerosene or mineral oil were recognized in the cheese. The temperature of wax application did not change the scores significantly (Table 8).

(2) *Trials Involving Waxes Produced Under Semi-commercial Conditions of Refining*

In the above instances where the experimentally produced waxes were scored low, the off-flavours were attributed to contamination resulting from batch operations. This indicated the necessity of securing wax produced from commercial-type equipment. Accordingly, a second series of waxes was prepared under semi-commercial conditions with specifications similar to waxes No. 274 and 278 mentioned in the earlier studies. This eliminated exposure of the waxes to contamination of rubber hose lines or to metals

TABLE 9.—PERFORMANCE OF SEMI-COMMERCIAL WAXES—AVERAGES OF DUPLICATE SLICES OF CHEDDAR CHEESE HELD FOR 32 DAYS AT 50° F. AND APPROXIMATELY 60 PER CENT R.H., WAXES APPLIED AT 220° F. FOR 6 SECONDS

Wax	Weight of wax applied (mg./cm.²)	Loss in weight (mg./cm.²)	Impact test	Conditions of freshly cut surface
274 A	32	12.9	N	Blisters, mould colonies, some cracks.
274 B	30	13.3	xxxx	8-10 small colonies, cracks.
274 B + 0.2 per cent A 200 polymer	34	11.2	N	Contracted from corners, blisters and cracks.
278 A	24	6.5	xx	Some blisters, 4 mould colonies.
278 B + 0.2 per cent A 200 polymer	27	7.6	x	Slight blistering, 6 small colonies, cracks.
278 B with 1.6 per cent oil	25	9.3	N	Slight blistering, 9 small colonies.
278 C	22	7.0	xx	Few blisters, 7 small colonies, cracks.
220 E*	36	7.6	N	Some blisters, mould colonies.
133° F.	18	8.6	xxxx	Slight blistering, 7 mould colonies.

* A commercial microcrystalline wax.

which might catalyze oxidation reactions. In addition trials were carried out with waxes containing a special plasticizer. This agent, Polymer A 200 (a polyethylene having a molecular weight of about 13,000), was added to two of the waxes at a rate of 0.2 per cent.

Inspection data on three wax samples without additives are given below:

Wax No.	Melting point ° F.	Oil content per cent	Penetration (ASTM D5-25)	Viscosity at 210° F.
274 A	148	2.9	23	64
278 A	131	1.4	27	48
278 C	131	0.9	24	48

Wax No. 274 A had properties identical to No. 274 used in the earlier experiments and may be regarded as a conventional melting point microcrystalline wax. No. 278 A and 278 C had melting points ten degrees above the No. 278 wax used previously. They also had a lower oil content but in other respects were similar. Shrinkage and flavour trials were conducted on the above waxes as well as on wax No. 278 B (containing 1.6 per cent oil). Included for comparative purposes were an imported microcrystalline wax (No. 220 E) and locally produced 133°-136° F. M.P. paraffin wax. Table 9 shows the results of these waxes when subjected to tests similar to those described under "Materials and Methods". (Only the 220° F. data are shown).

Wax No. 274 A gave results similar to No. 274 (previously tested) and showed good resistance to fracturing at both dipping temperatures. The tendency for some of the waxes to break and contract from the corners of the cheese resulted in increased shrinkage and mould growth. Good resistance to shattering was displayed by No. 274 A and 278 B. The shatter resistance of No. 274 B was greatly improved by the addition of the polymer. The commercial microcrystalline wax No. 220 E was slightly superior to all the others on the basis of all-round performance. Thicker wax films resulted in less shrinkage than in the previous trials.

The principal reason for studying the waxes produced on a semi-commercial scale was to evaluate the coatings from the standpoint of flavour imparted to the cheese; hence samples were submitted to a taste panel. The precision of the panel judgment was improved by (*a*) enlarging the number of evaluators to seven, and (*b*) agreeing on a score for the 133°-136° F. M.P. paraffin control sample prior to evaluating the specimens coated with the semi-commercial waxes.

Table 10 shows that several of the waxes again exhibited undesirable flavours, as indicated by the low scores obtained with wax No. 278 A as well as with the 278 B containing the added polymer. Reasons given for lower scores included off-flavours such as kerosene, oily or mineral oil. Although the waxes to which the polymer was added received lower-than-average scores the off-flavour could not be identified. The results indicate that in the production of these waxes some off-flavours were absorbed.

TABLE 10.—THE EFFECT OF SEMI-COMMERCIAL WAXES UPON CHEESE FLAVOUR—RESULTS OF 7 TASTERS—POSSIBLE RANGE 10 (EXCELLENT, NO OFF-FLAVOUR) TO 1 (HIGHLY OBJECTIONALBE)

Wax	Range of flavour for scores for waxing at:		Average score for waxing at:	
	190° F.	220° F.	190° F.	220° F.
133° F.	—	—	8.00*	8.00*
274 A	7–8	5–8	7.85	7.00
274 B	5–9	6–8	6.85	6.71
274 B + 0.2 per cent A 200 polymer	4–7	4–8	6.00	6.00
278 A	1–3	2–7	2.14	3.71
278 B + 0.2 per cent A 200 polymer	2–7	2–7	3.14	3.14
278 B with 1.6 per cent oil	7–9	6–8	7.57	7.00
278 C	6–9	6–9	7.28	7.43

* This standard score was agreed to by panel at commencement of scoring. Necessary difference between treatment means for two temperatures at 95 per cent confidence level = 0.9.

With No. 278 B containing the added polymer, flavour contamination appeared with the addition of the polymer. Sample No. 278 C as well as No. 278 B containing the 1.6 per cent oil gave greatly improved scores as compared to sample No. 278 in the previous series of tests. This clearly showed that flavour contamination can be largely prevented by large-scale processing.

DISCUSSION

The one phase of this problem which has received the most attention in this study has been shrinkage (loss in weight). Use of the flat cylindrical cheese slices, which had a large surface area per unit weight, afforded a good means of testing the moisture vapour barrier properties of the various waxes. Evaporation from large export size cheeses takes place at the same rate per unit area as with the small slices used in the experiment, but, because of the large weight per unit area relationship, shrinkage is not so apparent.

As a result of moisture evaporation from cheese surfaces during storage, the rinds become objectionably thick. This creates an economic loss of considerable magnitude to the industry and is partially the reason for the high mark-up in the retailing of cheese. This, together with the fact that thick-rinded cheese cannot be readily wire-cut for consumer packages, has resulted in the greater use of plastic films for wrapping natural cheese.

The moisture vapour barrier properties of none of the experimental waxes were as good as those of the commercially available cheese waxes of high oil content. The latter were all of a somewhat more fibrous character, and were probably truer types of microcrystalline waxes than any of the experimental samples, as indicated by their high melting points and high penetration values. Sample No. 274 (first series, Tables 1 and 2) closely approached these in performance and properties. Waxes No. 278 and 280 (Tables 1 and 2) as well as wax No. 278 B (Tables 9 and 10) containing the higher oil content (1.6 per cent) also gave good protection.

The dropping ball impact test was devised to measure the resistance to fracture of the wax films. In general, waxes which resisted fracture in this test gave good to excellent results in reducing moisture loss and preventing growth of surface moulds. The exceptions to this were the low melting point waxes which would be unsatisfactory due to their tackiness. Such waxes stick to shelving and to cheese boxes and make handling difficult. The tendency to fracture, as is indicated by the impact test, has been found the chief limitation of refined paraffins.

The studies dealing with dipping intervals and temperatures of wax application were conducted to obtain information on the much-disputed subject—correct waxing techniques. These experiments showed that high temperatures of application and long periods of immersion resulted in thin coats of wax and greatly increased shrinkage. This, together with the fact that overheating causes the wax to become discoloured and consequently a source of off-flavours, suggests that the application temperature of 220° F. is preferable.

SUMMARY AND CONCLUSIONS

A series of eight experimental, batch-produced petroleum waxes were compared with two paraffin waxes and five commercial waxes as to their physical properties and their performance as Cheddar cheese coating materials. An additional seven experimental waxes produced under semi-commercial conditions of refining were studied subsequently. The physical properties determined were melting point, oil content, penetration, viscosity at 210° F., and refractive index. Their possible utilization for coating cheese was assessed by (*a*) determining the shrinkage (loss in weight) of waxed slices of Cheddar cheese; (*b*) testing their resistance to fracturing by means of an impact test; (*c*) noting the occurrence of mould growth, blisters and other defects. Five of the eight batch-produced waxes (Samples No. 274, 275, 278, 279 and 280) and two of the four commercial microcrystalline waxes (Samples C and D) were superior to 133°-136° F. M.P. refined paraffin wax in resisting fracturing. From the standpoint of preventing weight loss (at 220° F. dipping temperature) the experimental waxes No. 275 and 280, as well as the commercial microcrystalline waxes (A, B, C, D and 220 E), were superior to the 133°-136° F. M.P. wax.

Immersing cheese in molten wax at temperatures of 250° F. and above, or holding in the wax for longer than 6 seconds, resulted in thin coatings and consequently high weight losses. Blending microcrystalline waxes with increasing increments of 133°-136° F. M.P. refined paraffin wax

produced films with less desirable properties. The performance of refined paraffin and of one experimental wax was not improved by the addition of mineral oil at rates up to 15 per cent.

Shrinkage trials carried out on shelf-cured, triplet size Cheddar cheese indicated that losses were considerably reduced over an 11-month period by dipping in refined paraffin followed by a second dip in a semi-commercially produced microcrystalline wax. This treatment afforded much better protection to the cheese surfaces.

Some of the experimental waxes imparted undesirable flavours to the surface of cheese. However, the data suggest that large-scale processing in a suitably designed plant would overcome this limitation.

ACKNOWLEDGEMENTS

This investigation was conducted with the aid of a grant from Imperial Oil Limited, Sarnia, Ontario.

REFERENCES

1. *Anon.* Installing waxing tanks and paraffining Cheddar cheese at the factory. Unnumbered circular, Can. Dept. of Agr., Ottawa. July, 1952.
2. Beattie, D. M. Butter and cheese in 1953. Can. Dairy & Ice Cream J. 33 : 30-32. 1954.
3. Bennett, H. Commercial waxes. Chemical Publishing Co., Brooklyn, N.Y. 1944.
4. Cunningham, J. D., O. R. Irvine, D. H. Bullock, and R. E. Emond. Efficacy of mould inhibitors as additives to paraffin coatings on Cheddar cheese. Rpt. Canadian Committee on Food Preservation, Nat. Res. Council of Can. (*Mimeographed*). 1953.
5. Doane, C. F. Methods and results of paraffining cheese. U.S. Dept. of Agr., Bur. Animal Ind., Cir. 181. 1911.
6. Swett, C. Waxes suitable for various types of cheese. Butter, Cheese, Milk Products J. 43, No. 7 : 28-29. 1952.
7. U.S. Patent No. 2, 187, 734.
8. U.S. Patent No. 2, 292, 323.
9. U.S. Patent No. 2, 322, 198.
10. U.S. Patent No. 2, 329, 470.
11. U.S. Patent No. 2, 527, 345.
12. U.S. Patent No. 2, 551, 087.
13. Van Slyke, Lucius L., and Walter V. Price. Cheese. 2nd ed. Orange Judd Pub. Co. Inc., New York. 1949.
14. Warth, Albin H. The chemistry and technology of waxes. Reinhold Pub. Co., New York. 1947.

THE DIGESTIBILITY OF DRIED GROUND SEAWEED MEAL BY THE LAYING HEN[1]

T. M. MacIntyre[2]

Experimental Farm, Nappan, N.S.

[Received for publication December 13, 1954]

ABSTRACT

Digestibility studies were carried out with laying hens on two species of seaweed. The two species studied, *Fucus vesiculosus* and *Ascophyllum nodosum*, are the rock weeds most abundant on the Nova Scotia coast. These two species of seaweed were poorly digested by the laying hen. In every case the addition of seaweed meal to the basal ration depressed the digestibility of all nutrients except fat.

The use of seaweed in the raw state, and as a meal when dried and ground, as a source of food for animals and poultry, has been under study for many years. However, there is still some doubt as to the nutritive value of seaweeds, in spite of the existence of a fair volume of published material on palatability (4, 5, 6, 13, 14, 15). This lack of knowledge is due partly to the fact that few well controlled digestibility trials have been carried out. Until very recently there was also a lack of appreciation of the large number of seaweed species available, the variations in chemical composition from species to species and the considerable seasonal variations within species.

Work to date has shown that seaweed cannot be regarded as a basic food. The value of seaweed meal is to be assessed, therefore, on the basis of its value as a supplementary feed, particularly its value as a source of minerals, vitamins, and available carbohydrates. Numerous feeding trials have shown that from 5 to 10 per cent of seaweed meal can be safely added to the diet of farm animals and poultry (1). Greater proportions frequently result in disturbed metabolism and very moist excrement.

A number of trials (12, 14, 15) have shown the digestibility of seaweed to be greater for ruminants than for swine. There is also some evidence that seaweed meal has a favourable effect on the nutritive value of the basic ration with which it is fed, although the reverse effect has also been noted, and in some cases a negative digestibility has been observed for protein (12, 15).

The object of the work reported herein was to study the digestibility, for poultry, of seaweed meal prepared from two different species of seaweed collected at different locations along the coast of Nova Scotia.

EXPERIMENTAL

The two rock weeds most abundant on the Nova Scotia coast, *Fucus vesiculosus* and *Ascophyllum nodosum*, were used in this study. The plants were harvested at low tide, partially dried in the sun and further dried in a steam-heated dryer. The dry material was ground in a Wiley mill to a fineness suitable for feeding. The species of seaweed, sites and times of collection, and proximate analyses of the seaweed meals used in this study are presented in Table 1.

[1] Contribution from Poultry Division, Experimental Farms Service, Canada Department of Agriculture.
[2] Senior Poultry Husbandman, Experimental Farm, Nappan, N.S.

TABLE 1.—SPECIES, SITES AND TIMES OF COLLECTION, AND PROXIMATE ANALYSES OF SEAWEED MEALS USED IN DIGESTIBILITY TRIALS

Species of seaweed	Site and time of collection	Moisture	Crude protein	Ether extract	Crude fibre	Ash	Nitrogen-free extract	Organic matter
		%	%	%	%	%	%	%
Mixed*	Near Digby, N.S., Oct., 1949	18.2	7.7	1.3	9.7	26.2	36.8	55.6
Ascophyllum nodosum	Hackett's Cove, May, 1953	12.8	3.9	5.6	3.3	16.5	57.9	70.7
Fucus vesiculosus	Herring Cove, May, 1953	12.8	12.1	3.6	4.4	17.4	49.7	69.9
Ascophyllum nodosum	Port Lorne, June, 1953	10.2	8.0	0.8	4.9	15.6	60.5	74.2
Fucus vesiculosus	Port Lorne, June, 1953	8.5	9.4	1.2	5.1	19.2	56.6	72.3

* Mostly *Ascophyllum nodosum* and *Fucus vesiculosus*.

The birds would not eat the seaweed meal when it was offered as the sole food. Accordingly, the meal was fed in admixture with a basal ration the digestibility of which had been previously determined. The ratio of basal ration to seaweed meal fed in each trial and the proximate analysis of each ration are shown in Table 2.

Seven trials were carried out with seaweed meal over a period of three years (1952-1954).

Six Barred Plymouth Rock laying hens, approximately one year old, were used on each trial. The birds were kept in individual metabolism cages similar to those described by Olsson and Kihlen (8). The experimental feeding period lasted 4 to 8 days and was preceded by a preliminary feeding period of 4 days. In the trials conducted in 1952 the feed was offered in the form of a dry mash and was kept before the birds from 9.00 a.m. until 4.00 p.m. Daily feed consumption was recorded by weighing back the unconsumed portion at the end of each day. In all other trials the feed was mixed with water and fed twice daily in the form of a moist mash in amounts which the birds would readily consume. Water was provided *ad libitum*. The duration of the trials and the average feed consumption per bird are shown in Table 3.

The excrement was collected on anodized aluminum trays placed under the metabolism cages. The excrement from each bird was collected daily at 8.00 a.m. and dried in an electrically heated oven at 80° C. At the termination of each trial, the total excrement from each bird was ground, mixed and sampled for analysis.

The feed and dried excrement were analysed for moisture, total nitrogen, ether extract, crude fibre and ash, by standard methods (7). The crude fecal nitrogen, as opposed to the urinary nitrogen, was deter-

TABLE 2.—COMPOSITIONS AND PROXIMATE ANALYSES OF RATIONS

Year	Trial number	Ration	Percentage composition of rations						
			Moisture	Crude protein	Ether extract	Crude fibre	Ash	Nitrogen-free extract	Organic matter
1952	1	Basal (1)	12.2	14.2	1.9	6.4	6.6	58.7	81.2
	2	70% Basal + 30% Seaweed Meal (*a*)	14.0	12.3	1.7	7.3	12.5	52.2	73.5
1953	3	Basal (1)	12.5	16.1	2.6	6.0	7.9	54.9	79.6
	4	70% Basal + 30% Ascophyllum (*b*)	12.6	12.4	3.5	5.2	10.5	55.8	76.9
	5	70% Basal + 30% Fucus (*c*)	12.6	14.9	2.9	5.5	10.7	53.4	76.7
	6	50% Basal + 50% Fucus (*c*)	12.6	14.1	3.1	5.2	12.6	52.4	74.8
1954	7	Basal (1)	9.6	18.9	2.2	4.5	7.9	56.9	82.5
	8	70% Basal + 30% Ascophyllum (*d*)	9.8	15.6	1.8	4.6	10.2	58.0	80.0
	9	90% Basal + 10% Fucus (*e*)	9.5	17.9	2.1	4.5	9.1	56.9	81.4
	10	70% Basal + 30% Fucus (*e*)	9.2	16.1	1.9	4.7	11.3	56.8	79.5

[1] Basal ration was in all-mash ration complete in all known nutrients.

Site and time of collection of seaweed:
(*a*) Near Digby, October, 1949.
(*b*) Hackett's Cove, May, 1953.
(*c*) Herring Cove, May, 1953.
(*d*) Port Lorne, June, 1953.
(*e*) Port Lorne, June, 1953.

TABLE 3.—NUMBER OF HENS, DURATION OF COLLECTION PERIOD, AND AVERAGE FEED CONSUMPTION

Year	Trial number	Ration	Number of hens	Collection period, days[1]	Av. feed consumed, gm.
	2	70% Basal + 30% Seaweed Meal	6	4	424
1953	3	Basal	6	5	480
	4	70% Basal + 30% Ascophyllum	6	5	500
	5	70% Basal + 30% Fucus	6	5	500
	6	50% Basal + 50% Fucus	6	4	207
1954	7	Basal	6	8	800
	8	70% Basal + 30% Ascophyllum	6	7	700
	9	90% Basal + 10% Fucus	6	8	960
	10	70% Basal + 30% Fucus	6	7	700

[1] The collection period was preceded by a preliminary feeding period of 4 days in each trial.

mined by the method outlined by Ekman, Emanuelson, and Fransson (2). The organic matter and nitrogen-free extract attributable to intestinal and urinary sources were calculated by the methods outlined by Olsson and Kihlen (8).

RESULTS AND DISCUSSIONS

The birds showed some reluctance to consume the rations containing seaweed meal when it was offered in the dry form. When the rations were moistened with water and offered as a moist mash, then the birds consumed them quite readily, with one exception. This exception was encountered in Trial 6 with the ration to which 50 per cent of seaweed meal (*F. vesiculosus*) had been added. In this trial considerable difficulty was experienced in getting the birds to consume the feed. There was no adverse effect on the taste or odour of the eggs when seaweed meal was fed.

The results of this work are summarized in Table 4. The addition of seaweed meal to the basal ration resulted in decreased digestibility coefficient for all nutrients, except fat in Trials 2 and 5. The digestibility of the seaweed meals tested was very low for all nutrients except fat.

The results indicate that these seaweed meals are poorly digested by the laying hen even when fed at levels as low as 10 per cent of the ration. These experiments do not support the view that seaweeds or seaweed products exert a beneficial influence upon digestion. Previous work at this Farm (4) showed that 10 per cent of seaweed meal was about the maximum which could be tolerated by hens and chickens. These results also showed that when seaweed meal was fed the birds consumed more feed. The addition of seaweed meal in excess of 10 per cent of the ration results in the consumption of excessive amounts of water and very soft droppings which may have an adverse effect on the digestibility of the basal ration with which the seaweed is fed. Work at Reading University (1) has shown that when 20 per cent of seaweed meal was fed the mineral metabolism of the birds was affected. It is possible that such a disturbance of mineral metabolism may adversely affect the digestion of other nutrients.

TABLE 4.—AVERAGE DIGESTIBILITY COEFFICIENTS OF BASAL RATIONS, BASAL RATIONS PLUS SEAWEED MEAL, AND SEAWEED MEALS[1]

Year	Trial number	Ration	Digestibility coefficients of basal rations and basal rations plus seaweed meals					Per cent change in digestibility coefficients of basal rations caused by addition of seaweed meals					Digestibility coefficients of seaweed meals				
			Organic matter	Crude protein	Ether extract	Nitrogen-free extract	Crude fibre	Organic matter	Crude protein	Ether extract	Nitrogen-free extract	Crude fibre	Organic matter	Crude protein	Ether extract	Nitrogen-free extract	Crude fibre
1952	1	Basal ration	75	82	71	75	15	—	—	—	—	—	—	—	—	—	—
	2	70% Basal + 30% Seaweed Meal	62	72	74	69	6	−17	−12	+ 4	− 8	−60	22	16	87	42	Neg.
1953	3	Basal ration	72	84	82	76	2	—	—	—	—	—	—	—	—	—	—
	4	70% Basal + 30% Ascophyllum	55	62	79	61	Neg.	−24	−26	− 4	−20	—	10	Neg.	76	29	Neg.
	5	70% Basal + 30% Fucus	61	69	85	65	2	−15	−18	+ 4	−14	—	33	23	90	34	Neg.
	6	50% Basal + 50% Fucus	43	52	73	47	Neg.	−40	−38	−11	−38	—	10	13	64	15	Neg.
1954	7	Basal ration	80	91	83	82	Neg.	—	—	—	—	—	—	—	—	—	—
	8	70% Basal + 30% Ascophyllum	58	64	79	64	Neg.	−27	−30	− 5	−22	—	2	Neg.	55	24	Neg.
	9	90% Basal + 10% Fucus	72	79	82	77	Neg.	−10	−13	− 1	− 6	—	3	Neg.	64	26	Neg.
	10	70% Basal + 30% Fucus	57	57	81	65	Neg.	−29	−37	− 2	−21	—	Neg.	Neg.	72	23	Neg.

[1] Values represent the mean of individual determinations from six birds.

The average digestibility coefficients reported in Table 4 suggest that seaweed meals are poorly digested by the fowl. However, as mentioned earlier, it was found necessary to mix the seaweed meal with a basal ration, the digestibility of which had been previously determined. The digestibility of the seaweed meal was then calculated by difference. It follows that any depression in the digestibility of the basal ration caused by mixing it with the seaweed would cause the digestibility of the seaweed meal to appear lower than it actually is. However, from the practical standpoint of using seaweed in poultry rations, it makes no difference whether its apparent low digestibility is inherent or due to a depressing effect on the digestibility of other components of the diet. Hence, for practical purposes, the digestibility figures obtained for seaweeds in these trials may be accepted.

The low digestibility coefficients observed may be due in fact to the nature of the species under study. These two species *F. vesiculosus*, and *A. nodosum*, have been found to be inferior to other species such as *Laminaria digitata* and *L. saccharina* (9) as a food for live stock. In feeding experiments with pigs, Ringen (11) found a negative digestibility of the proteins in *A. nodosum*. Furthermore, it has been shown (10) that *A. nodosum* and *F. vesiculosus* contains a substance which reduces the pepsin-digestibility of ordinary albumins. This would account for the low, and in some cases, negative digestibility of protein. The negative digestibility of crude fibre is not surprising in view of the inability of the fowl to handle this nutrient (3).

The poor digestibility coefficients obtained in this study indicate that these two species of seaweed must be regarded as a poor source of feed for poultry. The results of these digestibility studies, therefore, confirm results previously obtained in practical feeding trials (4).

ACKNOWLEDGEMENTS

The author wishes to acknowledge the co-operation and assistance of the following in carrying out this work: H. D. Smith, President, Nova Scotia Research Foundation, Halifax, N.S.; Constance MacFarlane, phycologist, Nova Scotia Research Foundation; laboratory technicians, Nova Scotia Research Foundation; H. S. Gutteridge, Chief of the Poultry Division, Central Experimental Farm, Ottawa, and J. R. Aitken, Senior Nutritionist, Poultry Division, Central Experimental Farm, Ottawa.

REFERENCES

1. Black, W. A. P. Seaweed as a stock food. Agriculture 60 : 126-130. 1953.
2. Ekman, P., H. Emanuelson, and A. Fransson. Investigations concerning the digestibility of protein in poultry. Annals of the Royal Agr. Coll., Sweden, 16 : 749-777. 1949.
3. Halnan, E. T. The architecture of the avian gut and the tolerance of crude fibre. Br. J. Nutr. 3 : 245-252. 1949.
4. MacIntyre, T. M., and M. H. Jenkins. Kelp meal in the ration of growing chickens and laying hens. Sci. Agr. 32 : 559-567. 1952.
5. Nilson, H. W., and J. W. Schaller. Nutritive value of agar and Irish moss. Food Research 6 : 461-469. 1941.
6. Nilson, H. W., and J. A. Wagner. Feeding tests with some algin products. Proc. Soc. Expt. Biol. Med. 76 : 630-635. 1951.

7. Official methods of analysis. Assoc. Off. Agr. Chem. 7th ed. Washington, D.C. 1950.
8. Olsson, N., and G. Kihlen. Edin's indicator method in digestibility experiments on poultry. Proc. 8th World's Poul. Congr., Copenhagen, 1 : 225-231. 1948.
9. Oy, Emil. Utilization of algae. Forente Nasjoners Vitenskapelige Konferanse. 1949.
10. Oy, Emil. The nitrogen compounds in seaweed. Tidsskrift for Kjemi, Berguesen Og Metallurgi., 82-84. 1951.
11. Ringen, J. The nutritive value of seaweed. Meld. Norg. Landburk. 19 : 451-541. 1939.
12. Senior, B. J., P. Collins, and M. Kelly. The feeding value of seaweeds. Econ. Proc. Royal Dublin Society 3, No. 19. 1946.
13. Sheehy, E. J. Seaweed as a food for farm stock. Dept. Agr. Eire, 44 : 5-9. 1947.
14. Sheehy, E. J., J. Brophy, T. Dillon, and P. O'Muineachain. Seaweed as a stock food. Econ. Proc. Royal Dublin Society 3, No. 12. 1942.
15. Woodward, N. Seaweeds as a source of chemicals and stock feed. J. Sci. Food Agr. 2 : 477-487. 1951.

SNOW AS A WATER REPLACEMENT FOR POULTRY[1]

L. A. Charette[2] and J. L. Tessier[3]

Experimental Station, Kapuskasing, Ontario

[Received for publication November 25, 1954]

ABSTRACT

An experiment was conducted during the winter months of 1948-49, 1950-51, and 1951-52 to determine whether snow could efficiently replace water in the feeding of laying birds. The birds on water had a significantly higher egg production as compared to birds receiving snow. The replacing of water by snow had no effect on feed consumption, egg size, or mortality. The pullets on water finished with a significantly better final weight at the end of each trial than those on snow. The results obtained demonstrated that snow could not be substituted for water for laying birds without adversely affecting winter egg production.

INTRODUCTION

A search of the literature revealed no specific information on the value of snow as a water replacement for laying birds. In reference to growing and laying turkeys, Marsden and Martin (3) stated that snow could not be depended upon to supply water in winter, although it might suffice for maintenance at times when it was impracticable or impossible to keep the water supply constantly open.

The supplying of cold water appears to have an effect on water consumption and egg production. The experiment of Kable and Fox (2), although lasting only 40 days, showed that water consumption was increased 5 per cent when warm water (45°-81° F.) was given as compared to cold water (37°-49° F.). These workers also mentioned that pullets drank about 25 per cent more warmed water than cold water during two days when the temperature remained below the freezing point all day. Beresford (1) found that there was little advantage in maintaining the drinking water at a temperature above 40° F., inasmuch as there was no apparent effect on egg production. However, a comparison of egg production of pens receiving water at 40°-50° F. and pens receiving water in which ice was allowed to form gave results decidedly in favour of the pens receiving the warm drinking water.

Wilson (4) observed, at an environmental temperature of 90° F., only a short interruption of egg production in Single Comb White Leghorns when water was withheld for 24 hours. When water was withheld for 48 hours, some of the pullets moulted, but later came back into production; the same was true for the 72-hour period. Furthermore, withholding water limited feed consumption drastically.

The Kapuskasing Experimental Station is situated at latitude 49° 25′, longitude 82° 28′, and at an altitude of 727 feet. Because of rigorous climatic conditions it is very difficult to prevent water inside the poultry house from freezing in winter. Therefore, an experiment was designed to investigate the relative effectiveness of snow as a replacement for water for

[1] Contribution from Poultry Division, Experimental Farms Service, Canada Department of Agriculture.
[2] Agricultural Research Officer.
[3] Poultryman.

laying birds and was carried out during three winters. During the time this project was conducted, temperatures of −30° to −42° F. were recorded on several occasions. Under such conditions, temperatures of 0° F. inside the poultry pens were reported at times. Night temperatures which were below freezing point inside the pens have been recorded for continuous periods as long as 21 days. However, during these periods, the temperature would usually rise above freezing during the daytime. On occasions, inside temperatures have remained below freezing for periods as long as 100 consecutive hours.

MATERIALS AND METHODS

Barred Plymouth Rock chicks from the same parent stock were hatched in April and early May and were reared in brooder pens until mid-June when they were placed on an alfalfa pasture. The birds were raised under identical conditions and were housed in mid-October. At that time, they were assigned at random to three laying houses. Each house was equipped with a different ventilation system (straw loft, flue, and modified Sanctuary) and each had two pens accommodating 50 birds. The pullets in each house were divided into two groups; one group served as a control (water was supplied), while the other group received snow instead of water. Since snow was a limiting factor, the duration of the trial varied each year. In 1948, the experiment was started on December 27 and lasted for 112 days. In 1950 and 1951, it started on November 1 and continued for 161 and 158 days, respectively.

An 18 per cent dry laying mash was fed *ad libitum* to both groups from the start of the experiment until the first week of January, when this was replaced by an 18 per cent hatching mash. A mixture of whole grains (equal parts oats, barley, and wheat) was spread on top of the mash at 4 p.m. Oyster shell was kept before the birds at all times and no grit was supplied. Cod liver oil (850A-85D) at the rate of 1 ounce per 50 birds was given daily throughout the experiment. The birds always had access to a sufficient supply of water or snow. In order to prevent the water from freezing in the pens where water was provided, a 6½-gal. fountain resting on an electric base was used. The heat was supplied by a 60-watt cone heater. This usually kept the water between 35° and 45° F., but on cold nights a thin layer of ice could be observed on top of the water inside the fountain.

A record of feed consumption, egg production, egg size, body weight, and mortality was maintained. Feed consumption data were kept by weighing the feed used in each pen and weighing the unconsumed feed at the end of each 28-day period of recording egg production. The average feed consumption per bird for each pen was calculated on a bird day basis at the end of each period after allowance for mortality had been made. Records of individual egg production were maintained throughout the experiment by trap-nesting 6 days per week. This was converted to a 7-day production by using the conversion factor 1.1667. The egg size was determined by weighing individually all the eggs laid in one day at the end of each 28-day period of November, December, and January and

TABLE 1.—MEAN PRODUCTION AND FEED CONSUMPTION DATA FOR BIRDS ON TWO TREATMENTS, SNOW VS. WATER, IN THREE DIFFERENT YEARS

	Trial 1 (1948-49)		Trial 2 (1950-51)		Trial 3 (1951-52)	
	Water	Snow	Water	Snow	Water	Snow
Number of days on test	112	112	161	161	158	158
Average number of birds per pen at start of experiment	49.7	50.0	50.0	49.7	50.0	49.7
Average number of birds per pen at end of experiment	47.3	47.7	46.7	47.3	49.7	47.7
Number of bird days per pen	5494.0	5540.7	7745.3	7735.7	7854.7	7665.3
Average age of birds at start of experiment (days)	257.1	253.2	191.7	194.9	203.3	205.0
Per cent mortality	4.7	4.7	6.7	4.7	0.7	4.0
Average initial body weight (lb.)	6.14	6.03	5.85	5.78	5.63	5.51
Average final body weight (lb.)	6.16	5.76	6.14	5.96	6.20	5.80
Egg production—						
Number of eggs per pen	3661.3	3443.7	4095.3	3536.7	4938.3	4021.7
Number of eggs per bird daily	0.67	0.62	0.53	0.46	0.63	0.52
Average number of eggs per bird during test	74.4	68.7	83.8	73.3	97.9	81.7
Daily feed consumption—						
Pounds of mash per bird	0.15	0.15	0.18	0.17	0.18	0.17
Pounds of scratch grain per bird	0.18	0.19	0.16	0.16	0.16	0.16
Pounds of oyster shell per bird	0.016	0.016	0.010	0.010	0.014	0.012
Ounces of cod-liver oil per bird	0.009	0.009	0.018	0.018	0.016	0.017
Feed efficiency for egg production—						
Number of eggs per 100 lb. feed	188.7	178.3	152.7	135.7	178.3	151.4
Average egg size (gm.)	62.76	62.47	59.99	59.80	60.70	60.60

for two consecutive days at the end of each week during February and March. Each bird was weighed at the beginning of the experimental season, and each surviving bird was weighed again at the end of the trial.

RESULTS

The effects of each treatment are summarized in Table 1. The analysis of variance for egg production on survival basis, egg size, initial body weight, and final body weight is presented in Table 2. Under the conditions of this experiment, there was a highly significant difference ($P < 0.01$) in the number of eggs laid between the birds receiving water to drink and the birds receiving snow. In each of the three years when this project was carried out, the egg production was lower when snow replaced water, namely by 7.5 per cent, 13.2 per cent, and 17.5 per cent, respectively. Since there was no significant difference in egg size between treatments (Table 2), the differences in total volume of eggs produced were similar.

As shown in Table 2, there existed no significant difference between treatments in initial body weight. The higher initial weight of the birds in Trial 1 (Table 1) was due to the fact that this trial started on December 27, whereas in the other two years the trials started on November 1 and the birds in Trial 1 were therefore older at the beginning of the test. The birds receiving water showed a higher final body weight at the end of each trial than those receiving snow. This difference was significant at the 1 per cent probability level in Trials 1 and 3, and at the 5 per cent probability level in Trial 2. In Trial 1, the control birds maintained body weight, whereas the birds receiving snow showed a loss in weight. In Trials 2 and 3, the birds on both treatments gained weight, but the increase was greater for those receiving water.

The replacing of water by snow had no effect on the amount of feed consumed or on mortality as suggested by the data in Table 1. However, because of the lower egg production from the birds receiving snow, a difference in feed efficiency existed in favour of the birds receiving water.

DISCUSSION

The results of this experiment indicate that there is a definite advantage to keep water before the birds even though it might involve extra attention to prevent this water from freezing. Although Marsden and Martin (3) made reference to growing and laying turkeys, their statement that snow cannot be depended upon to supply water is in agreement with the results obtained during the three seasons this test was undertaken. As shown by Beresford (1), birds will consume the same amount of water at 50° F. as at 40° F., once they have been accustomed to the warmer water for a few days. He also reported that pens of birds receiving water in which ice was allowed to form gave an egg production record decidedly in favour of the pens receiving water at 40°-50° F. The findings of the above worker are not directly comparable with the work reported herein since no snow was given to his experimental birds. The results obtained at Kapuskasing are comparable to these in that the limiting factor, as far as the performance

TABLE 2.—ANALYSIS OF VARIANCE

Source of variation	D.F.	Mean squares for			D.F.	Mean squares for initial body weight
		Egg production	Egg size	Final body weight		
Trial 1 (1948-49)						
Houses	2	1881.26**	23.03	2.36*	2	0.41
Treatments	1	2746.55**	7.98	11.10**	1	0.91
Remainder (error)	279	196.11	14.28	0.51	295	0.48
Total	282				298	
Trial 2 (1950-51)						
Houses	2	4437.30**	0.38	0.27	2	0.13
Treatments	1	9940.01**	28.73	2.13*	1	0.39
Remainder (error)	274	435.39	17.38	0.47	295	0.42
Total	277				298	
Trial 3 (1951-52)						
Houses	2	6351.90**	25.36	0.42	2	1.60**
Treatments	1	16604.33**	9.01	11.59**	1	1.01
Remainder (error)	286	593.23	19.32	0.37	295	0.29
Total	289				298	

* Significant at 5 per cent probability level.
** Significant at 1 per cent probability level.

of the birds is concerned, was probably the total amount of water ingested, either in the form of snow or as water at a lower temperature than the control.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to F. X. Gosselin, Superintendent, Experimental Station, Kapuskasing, Ontario, for his encouragement; to H. S. Gutteridge, Chief, Poultry Division, Central Experimental Farm, Ottawa, Ontario, for his direction and advice prior to and during the course of the experiment; and to A. S. Johnson, Poultry Division, Central Experimental Farm, Ottawa, Ontario, for reviewing the manuscript.

REFERENCES

1. Beresford, H. Stock tank and poultry water heaters. Agr. Eng. 11 (8). 1930.
2. Kable, G. W., and F. E. Fox. Electric water heaters for poultry. Oreg. Expt. Sta. Cir. 81. 1927.
3. Marsden, S. J., and J. H. Martin. Turkey management. 5th ed. The Interstate, Danville, Ill. 1949.
4. Wilson, W. O. Response of laying hens under thermal stress to cold drinking water and lack of water. Poul. Sci. 30 : 933. 1951.

COORDINATED SOIL-PLANT ANALYSIS

III. EXCHANGE EQUILIBRIA IN SOIL SUSPENSIONS AS POSSIBLE INDICATORS OF POTASSIUM AVAILABILITY[1,2]

D. C. MacKay[3] and W. A. DeLong[4]

Macdonald College, Que.

[Received for publication December 20, 1954]

ABSTRACT

A technique of equilibration between solutions of low hydrogen ion concentration and the exchangeable cations of soils was applied to the investigation of the ease of release of potassium from a variety of soils differing considerably in the compositions of their suites of exchangeable cations. The hydrogen ion concentrations used ranged downward to those produced by the addition of water only to the soil samples. The amounts of potassium released were estimated by flame photometry.

Highly significant positive correlations were found between the quantities of potassium released by equilibration of inorganic soils with water and the amounts of this element present in the leaf tissue of corn plants grown on the same soils. The two samples of organic soils examined in this way did not show this relationship.

The evaluation of available soil potassium by determination of exchangeable values has often given unsatisfactory results, and it is generally assumed that ionic activity, at field moisture, should provide the best indication of the quantities offered for plant use at any given time. Although considerable progress has been made in measuring individual ion activities in clay-water suspensions, in the presence of a limited number of ions (7, 8, 11, 15), the early application of these direct procedures for diagnostic purposes appears doubtful.

A method involving the competitive exchange of potassium and other cations for small amounts of hydrogen ions (1.66 m.e. per 100 gm. of soil) was first suggested by Bray (1). The technique was designed to measure the relative ease of release of various ions from different soils. This and similar procedures (2, 3, 12, 13) have apparently provided useful information in estimating crop requirements. An appealing feature of the approach is the similarity of the proposed reaction to the most widely accepted mechanism for initial ion absorption by plant roots (14). The obvious objection to such procedures is the arbitrary selection of the concentration of replacing ions to be used. However, Marshall (10) has pointed out that the concentrations of ions released with increasing dilutions should approach ever more closely their activities; and Wiklander (20, 21) has shown with synthetic resins that the ratios of released ions approached constant values with decreasing concentration of replacing ions. Presumably these values would correspond with the ratios of the ion activities, and determination of potassium concentration at the constancy point should give a relative measure of the availability.

---

[1] Contribution from the Department of Chemistry, Macdonald College (McGill University). Presented before Soils Section, Agricultural Institute of Canada, Macdonald College, Que., June 23, 1954.

[2] Macdonald College Journal Series No. 363, Macdonald College, Que.

[3] Formerly holder of American Potash Institute Fellowship; now Agricultural Research Officer, Experimental Station, Kentville, N.S.

[4] Professor of Chemistry.

The purpose of this investigation was to examine the applicability of various concentrations and types of proton donors for exchange equilibria reactions, by comparing, through field-collected samples, the potassium status of soils and corn plants grown thereon.

MATERIALS AND METHODS

The soil and corn leaf samples were collected from the Macdonald College Seed and Stock Farms. The former area is operated as a seed production unit and has been under uniform cropping and management practices for some 20 years, while the latter is operated to supply hay, pasture and corn silage for the College herd, with small grains being grown on the remaining acreage. This area has been subjected to a greater variety of fertilizer and manurial treatments in keeping with the herd requirements.

An account of the pertinent geology of the area and descriptions of the Seed Farm soil types have been given previously (5). The Stock Farm types included the Chicot fine sandy loam and the St. Benoit light sandy loam of the Brown Podzolic Group; the St. Amable loamy sand, a Ground Water Podzol; the St. Laurent clay, a Dark Grey Gleisolic soil; and the Muck and St. Zotique silt loam, which represent the Bog and Half Bog Groups, respectively.

The corn varieties sampled were DeKalb 240 and Pioneer 355 from the Stock Farm, and Quebec 28 from the Seed Farm. The former varieties are both medium maturing. slight tillering vigorous double cross dent hybrids for silage corn; the latter is a mass selection flint type, producing small, low-yielding plants that are detasselled to become the female parent in the production of Algonquin hybrid seed.

The sampling procedure and the flame photometric methods of analyses employed have been described earlier (5, 6). The sources of hydrogen ion examined were dilute HCl, H-saturated cation exchange-resin, carbonated water, and carbonate-free distilled water. The suitability of each was evaluated by deriving the linear correlation coefficients for the relationship between released soil potassium and leaf potassium.

Carbonate-free distilled water equilibration was tested on the largest scale. In this scheme a mixture of 50 gm. of soil and 150 ml. of recently boiled distilled water (cooled under soda-lime protection) was agitated in a stoppered flask for one hour on a rotary shaker, and was filtered immediately through a very retentive paper (Whatman No. 3) with the aid of suction. A 100-ml. aliquot was evaporated to a small volume, the organic matter destroyed by digestion with aqua regia, and the salts converted to chlorides. Finally, the residue was dissolved in 10 ml. of 0.2 N HCl for the estimation of potassium at the flame photometer.

Equilibration of a number of soils with dilute HCl at a theoretical pH of 4.7 was performed, after first establishing a concentration at which the ratio of calcium to potassium, released into solution, approached a constant value. This treatment involved a procedure similar to that adopted for water equilibration. Ten-gm. samples of soil were shaken with 30 ml. of solution for two hours, then centrifuged, and an aliquot treated in the usual way for flame photometric determination of cations.

For the experiments with carbonated water, saturation of 30-ml. portions was carried out at atmospheric pressure by progressive passage of carbon dioxide (from a commercial cylinder) through a series of 40-ml. centrifuge tubes at 70° F. and 65 per cent relative humidity. When a constant pH was reached (about 4.0), 10 grams of soil were quickly added, the tubes corked and shaken for two hours, and finally the solutions were centrifuged. Potassium was determined in an aliquot, after evaporation and conversion to chlorides.

Exchange of metallic cations for hydrogen from a weakly acidic cation exchange resin (Amberlite 1R C-50) was also investigated as it was felt that this approach, more nearly than the others, simulated the generally assumed mechanism of the plant root during the first stages of ion absorption. However, difficulty was experienced in perfecting a technique which would prevent contact of the resin with the colloidal soil particles and still permit attainment of an equilibrium condition through the liquid phase. Placing the resin in sintered glass crucibles ("M" porosity) appeared to fulfil the latter requirement, but this also allowed some of the colloids to pass through. In this event, part of the solid matter apparently became associated with the resin as complete potassium elution was effected very slowly in a way quite foreign to the normal behaviour of the pure resin.

RESULTS AND DISCUSSION

The results of an experiment to establish a less arbitrary acid concentration for the equilibrium reactions with soils are given in Table 1. In common with Wiklander's results with synthetic exchange resins (21), they indicate that decreasing additions of hydrogen ions released concentrations of potassium and calcium whose ratios approached constant values. Incidentally, the acid addition at which this point was reached was very much less than that employed in Bray's study (1).

An acid solution of slightly lower concentration than the lowest of Table 1 was used to test the theory that equilibration of such a solution with different soils should reveal the relative availability of potassium for plants. Mean potassium values for two groups of Seed Farm soils, repre-

TABLE 1.—CATIONS RELEASED (M.E./100 GM.) BY EQUILIBRATING SOLUTIONS OF VARIOUS CONCENTRATIONS OF HCl

H^+ added (m.e./100 gm. of soil)	Sample "A"		Sample "B"	
	K	Ca/K	K	Ca/K
100	0.332	19.6	0.160	39.4
20	0.267	22.2	0.120	47.0
4	0.166	17.5	0.074	39.6
0.8	0.085	8.7	0.033	21.9
0.16	0.071	4.9	0.024	14.5
0.032	0.060	4.9	0.021	13.8

senting collections in 1950 and 1951, each of which includes three soil types, are presented in Table 2. In addition, this table presents the results obtained from equilibration with the other sources of hydrogen ions. Although the concentrations released were generally inversely related to the concentrations of acid used, that released by the carbonate-free water treatment was higher than expected.

The correlation coefficients, showing the relationship between soil potassium released and percentage leaf potassium for the individual samples, are given in Table 3. The high correlations for the equilibration procedures are especially noteworthy in the 1951 samples, the carbonate-free distilled water being superior. The relationship between exchangeable potassium and leaf potassium gave a very poor correlation in this year. The 1950 samples, although yielding a good correlation for the exchangeable potassium determination, also gave a highly significant correlation for the water equilibrated method.

In view of the encouraging results obtained from the water-equilibration procedure with the Seed Farm soils, the method was given a more extensive trial on samples from additional soil types and from fields with less uniform cropping management. When the results from the four mineral soil types from the Stock Farm were grouped, the correlation coefficient relating the soil and leaf potassium (Table 4) was highly significant for the water released potassium, while that for exchangeable

TABLE 2.—POTASSIUM RELEASED (M.E./100 GM. OF SOIL) FROM SEED FARM SAMPLES USING VARIOUS PROCEDURES

Procedure	Mean values, 10 samples each	
	1951 collection	1950 collection
Leaching with 1 N. NH_4Ac	0.208	0.266
HCl equilibration (2×10^{-5} N)	0.0180	0.0256
H_2CO_3 equilibration:		
1 atmos. CO_2	0.0318	0.0460
1/5 atmos. CO_2	0.0238	—
Carbonate-free dist. water equilibration	0.0193	0.0287

TABLE 3.—CORRELATION COEFFICIENTS FOR RELATIONSHIP BETWEEN LEAF POTASSIUM (TOTAL) AND SOIL POTASSIUM RELEASED BY VARIOUS REAGENTS

Procedure	Correlation coefficients	
	1951 samples	1950 samples
NH_4Ac leaching	0.005	0.867**
HCl equilibration	0.642*	0.913**
H_2CO_3 equilibration:		
1 atmos. CO_2	0.172	0.725*
1/5 atmos. CO_2	0.619*	—
Dist. water (CO_2 free) equilibration	0.905**	0.883**

* and ** Indicate significance at "p" of 0.05 and 0.01, respectively.

potassium was not significant. However, the comparisons within soil types were usually slightly poorer with the former method, probably because of larger analytical errors in the determination of the small concentrations of potassium. The two areas of highly organic soils gave data for both methods, which were inconsistent with those from the other types, since the potassium values from the soil samples were very high, while those from the leaf samples were the lowest of all areas (Table 5). The abnormally high water-equilibrated values are thought to be due to removal of potassium ions in association with dissolved organic matter, these being liberated when the solutions were digested and concentrated. It might be possible to evolve a method for removing these soluble organic fractions and thus permit a more accurate evaluation of released potassium for these soils.

TABLE 4.—CORRELATION COEFFICIENTS SHOWING THE RELATIONSHIP BETWEEN LEAF POTASSIUM CONTENT AND SOIL POTASSIUM AS DETERMINED BY TWO METHODS

Area sampled	Number of samples	Correlation coefficients	
		Water-equilibrated K	Exchangeable K
Four soil types	58	0.535**	−0.146
Chicot	16	0.654**	0.679**
St. Benoit	12	0.492	0.740**
St. Amable	12	0.551*	0.620*
St. Laurent	18	0.056	−0.507*
Muck	20	0.305	0.086
St. Zotique	4	0.918*	0.946*

* and ** Indicate significance at "p" of 0.05 and 0.01, respectively.

TABLE 5.—MEAN VALUES FOR LEAF AND SOIL POTASSIUM IN SAMPLES FROM SEVERAL AREAS

Area sampled	Leaf potassium (per cent)	Soil potassium (m.e./100 gm.)	
		Water equilibrated	Exchangeable
1950 seed farm	3.09	0.0287	0.266
1951 seed farm	2.53	0.0193	0.208
Grenville calcareous	2.04	0.0124	0.200
Chicot (St.)	2.33	0.0210	0.185
St. Benoit (St.)	2.24	0.0204	0.156
St. Laurent (St.)	1.96	0.0134	0.330
St. Amable (St.)	1.65	0.0170	0.127
St. Zotique (St.)	1.44	0.0253	0.197
Muck (St.)	0.95	0.0492	0.281

TABLE 6.—EQUATIONS FOR REGRESSION OF LEAF TISSUE POTASSIUM (Y) ON SOIL POTASSIUM (X), AND CALCULATED "CRITICAL" SOIL LEVELS

Area sampled	Regression equation	"Critical" soil level (m.e./100 gm.)
Exchangeable potassium		
Stock farm (58)*	$Y_e = 2.18 - 0.59X$	Neg.
Seed farm (40)	$Y_e = 1.20 + 5.55X$	0.08
Muck (20)	$Y_e = 0.66 + 1.04X$	0.95
Water-equilibrated potassium		
Stock farm (58)	$Y_e = 1.44 + 34.4X$	0.006
Seed farm (40)	$Y_e = 1.49 + 51.0X$	0.003
Muck (20)	$Y_e = 0.88 + 1.4X$	0.61

* Figures in brackets refer to number of samples.

TABLE 7.—CATION COMPOSITION OF SOIL SOLUTIONS REPORTED IN THE LITERATURE (RECALCULATED TO M.E/100 GM. OF SOIL)

Investigators*	Nature of sample	Per cent moisture	Cations present	
			K	Ca/K
Schloesing (16)	—	19.1	0.0028	64.2
Reitemeier and Richards (17)	Clay loam pH − 8 +	14.2	0.162	38.8
		20.0	0.188	44.2
		32.5	0.209	44.0
Vlamis (19)	Unfertilized, acidic	20.0	0.008	2.5
	Unlimed, acidic	20.0	0.014	9.7
	Same soil, limed	20.0	0.012	17.8

* All workers used the displacement method, essentially as outlined by Burd and Martin (4).

The leaf percentages given in Table 5 suggest that all samples, except those from the organic soils and the St. Amable area, are well above the critical level of 1.65 per cent indicated from the data reported by Tyner (18)†. Soil values corresponding to this critical leaf percentage have been estimated from the regression equations (Table 6). Several features of these calculations may be emphasized:

(1) The regression equations for the exchangeable potassium values are extremely inconsistent for the two groups of samples, and the critical level for the Stock Farm is a negative value.

(2) The water-equilibration method yielded regression equations of different slopes, but this might be expected in view of the different types of corn grown on the two farms. The difference in slope also resulted in a lower critical soil level for the Seed Farm areas.

(3) The regression equations and critical levels obtained from the Muck area, with both methods of determination, are quite different than those from the other areas— as to origin and slope of the regression lines, and also in the calculated critical levels. Extremely high Ca/K values were obtained for these areas; this may help to account for the depressed potassium uptake.

† Tyner proposed 1.30 per cent as a tentative critical level, but the figure quoted above would appear to correspond more closely to the term "critical level", as originally defined by Macy (9).

It may be of interest to compare the cation compositions of the equilibration solutions obtained here with those reported in the literature for solutions displaced from soils at or near field moisture (4, 16, 17, 19). When such data are recalculated to a comparable basis (Table 7), it may be seen that the absolute quantities of potassium removed in the soil solutions were of the same order as those obtained in the present study by water-equilibration (Table 5). Further, the calcium-potassium ratios recorded in Table 1 compare favourably with those of the acid soils investigated by Vlamis, and the ratios from Reitemeier and Richards' data are relatively constant for a soil at various moisture percentages. Thus, it appears that the equilibration technique gives a fairly reliable indication of the composition of the soil solution.

These results would suggest that a method involving an equilibrium reaction with a weak proton donor offers attractive possibilities for assessing the relative potassium availability of soils. In common with any diagnostic procedure, its practical application would require calibration with crop yields under field conditions.

REFERENCES

1. Bray, R. H. Ionic competition in base exchange reactions. J. Amer. Chem. Soc. 64 : 954-963. 1952.
2. Brown, D. A. Cation exchange in soils through the moisture range saturation to wilting percentage. Soil Sci. Soc. Amer. Proc. 17 : 92-96. 1953.
3. Brown, D. A., and W. A. Albrecht. Complementary ion effects in soils as measured by cation exchange between electrodialyzed hydrogen clay and soils. Soil Sci. Soc. Amer. Proc. 15 : 133-138. 1951.
4. Burd, J. S., and J. C. Martin. Water displacement of soils and the soil solution. J. Agr. Sci. 13 : 265-295. 1923.
5. DeLong, W. A., D. C. MacKay, and H. A. Steppler. Coordinated soil-plant analysis. I. Nutrient cations. Soil Sci. Soc. Amer. Proc. 17 : 262-266. 1953.
6. MacKay, D. C., and W. A. DeLong. Coordinated soil-plant analysis. II. Accuracy and precision of flame photometric methods for cation analysis. Can. J. Agr. Sci. 34 : 451-459. 1954.
7. MacLean, E. O. Interrelations of potassium sodium and calcium as shown by their activities in a beidellite clay. Soil Sci. Soc. Amer. Proc. 15 : 102-106. 1951.
8. MacLean, E. O. The effect of humus on cationic interactions in a beidellite clay. Soil Sci. Soc. Amer. Proc. 16 : 134-136. 1952.
9. Macy, P. The quantitative mineral requirements of plants. Plant Physiol. 11 : 749-764. 1936.
10. Marshall, C. E. The exchangeable bases of two Mississippi soils in relation to composition of four species. Miss. Agr. Expt. Sta. Res. Bull. 385. 1944.
11. Marshall, C. E. The activities of cations held by soil colloids and the chemical environment of plant roots. The mineral nutrition of plants, Chapter 3. University of Wisconsin Press, Madison, Wis. 1951.
12. Mehlich, A. Cations in plants and soil extracts. Soil Sci. 62 : 393-409. 1946.
13. Mehlich, A., and J. F. Reed. Effect of cation exchange properties of soil on the cation content of plants. Soil Sci. 66 : 289-306. 1948.

14. Overstreet, R., and L. Jacobson. Mechanisms of ion absorption by roots. Ann. Rev. Plant Physiol. 3 : 189-206. 1952.
15. Peech, M., and A. D. Scott. Determination of ionic activities in soil-water systems by means of the Donnan membrane equilibrium. Soil Sci. Soc. Amer. Proc. 15 : 115-119. 1951.
16. Russell, E. J. Soil conditions and plant growth. 8th ed., revised by E. W. Russell. Longmans, Green and Co., London. 1950.
17. Reitemeier, R. F., and L. A. Richards. Reliability of the pressure-membrane method for extraction of soil solutions. Soil Sci. 57 : 119-135. 1944.
18. Tyner, E. H. The reaction of corn yields to leaf nitrogen, phosphorus and potassium content. Soil Sci. Soc. Amer. Proc. 11 : 317-323. 1946.
19. Vlamis, J. Acid soil infertility as related to soil-solution and solid-phase effects. Soil Sci. 75 : 383-394. 1953.
20. Wiklander, L. Equilibria in ion-exchange. I. Influence of the proportions of the exchanging ions. Acta Agriculturae Scand. 1 : 190-202. 1951.
21. Wiklander, L., and E. Nilsson. Equilibria in ion exchange. II. Influence of the mode of replacement. Acta Agriculturae Scand. 2 : 197-209. 1952.

FURTHER STUDIES ON THE CHEMOTHERAPY OF DISEASES OF THE HONEYBEE[1]

H. Katznelson[2], C. A. Jamieson[3] and G. H. Austin[3]

Science Service and Experimental Farms Service, Canada Department of Agriculture, Ottawa, Canada

[Received for publication December 23, 1954]

ABSTRACT

The minimum dosage of terramycin or sulphadiazine which would protect a colony of bees from American foulbrood for an entire season was 250 mg. Terramycin lost its effectiveness when stored 6 months in honey. The antibiotic achromycin also was shown to be effective against American foulbrood.

Of various substances tested, Fumidil B (containing the antibiotic fumagillin) was the most effective in controlling Nosema disease in adult bees in cages. Anisomycin, another antibiotic, reduced infection 50 per cent, but Win 5047, Vi-oxaline and erythromycin were ineffective.

The work reported in this paper was carried out during the late spring and summer of 1954 as part of a long-term project on the control of diseases of the honeybee. This project is concerned primarily with testing new drugs and antibiotics as they become available; determining suitable dosages, stability and the most effective means of application; and with related problems. During the course of this work, it was discovered, for example, that the antibiotic fumagillin could control *Nosema apis*, a protozoan disease of adult bees (5); that terramycin was effective against American foulbrood (AFB) (6) and that this compound and streptomycin were efficacious in controlling European foulbrood (EFB) (8). As new drugs and antibiotics are discovered they will be tested in a similar manner in attempts to obtain one or more which will be completely effective in small amounts, stable over an extended period of time, and perhaps able to control more than one disease. Terramycin fulfils two of these criteria but, as will be shown, is not very stable. Sulpha drugs such as sulphadiazine on the other hand, though quite stable (7), appear to inhibit only AFB.

AMERICAN FOULBROOD

Stability of Terramycin in Honey

In December, 1953, 250 mg. terramycin was added to one 8-lb. pail of honey and 500 mg. to a second. Both pails were stored in a warm room to simulate hive temperature. In June, 1954, both pails, along with a pail of untreated honey, were inoculated with a heavy suspension of spores equivalent to one AFB scale per pail. The pails of inoculated honey were then fed to new colonies installed on dry comb. Within one month's time all three colonies showed evidence of AFB, whereas other colonies, receiving fresh terramycin and treated similarly, did not. Thus it appears that terramycin is not stable under the conditions of these experiments. This

[1] Contribution No. 382 from the Bacteriology Division, Science Service.
[2] Bacteriology Division, Science Service.
[3] Apiculture Division, Experimental Farms Service.

TABLE 1.—THE EFFECT OF DIFFERENT AMOUNTS OF SULPHADIAZINE AND TERRAMYCIN ON AMERICAN FOULBROOD[1]

Compound (mg. per 8-lb. pail honey)	Examination of colonies					
	21 days		50 days		93 days	
	C[2] 1	C 2	C 1	C 2	C 1	C 2
Sulphadiazine						
500	−	−	−	−	−	−
250	−	−	−	−	−	−
125	−	−	+	−	+	+
62	+	−	+	−	+	+
0	+	+	+	+	+	+
Terramycin						
250	−	−	−	−	−	−
125	−	−	−	−	+	−
62	−	−	+	−	+	+
31	−	−	+	−	+	+

[1] − not infected.
+ infected.
[2] C = colony.

observation was borne out further by a report from the field that a colony which had been fed inoculated and terramycin-medicated honey in 1953 and had been free from AFB for the entire season showed evidence of considerable infection in 1954. Sulpha drugs usually remain effective under similar conditions.

Effective Dosage of Sulphadiazine and Terramycin

To determine effective dosage, a range of concentrations of sulphadiazine and terramycin was added to 8-lb. pails of 50 per cent sugar solution, each pail inoculated with spores from one AFB scale and the syrups fed to duplicate colonies of bees. Examinations at intervals throughout the summer revealed that by the end of the season AFB had developed in all colonies receiving less than 250 mg. of sulphadiazine or terramycin (Table 1).

Miscellaneous Tests

Experiments were conducted also to determine whether 500 mg. of sulphadiazine or 250 mg. of terramycin could protect colonies against 10 times the amount of inoculum used in the preceding tests. Complete protection was achieved with these amounts for the entire season.

Two new antibiotics, achromycin and pyridinethione*, were tested in 250 mg. amounts in 10-lb. pails of sugar syrup, each containing spores from one AFB scale. Achromycin was effective in controlling this disease throughout the summer whereas pyridinethione was not.

* Obtained from Lederle Laboratories and The Squibb Institute for Medical Research, respectively.

TABLE 2.—ANTI-NOSEMA ACTIVITY[1] OF VARIOUS ANTI-PROTOZOAN AGENTS

Treatments	Amounts used (mg./30 ml. syrup)	Per cent infection
Uninoculated	—	0
Inoculated	—	93
Inoculated + Fumidil B	5	3
Inoculated + Fumidil B	50	2
Inoculated + PA 106	30	48
Inoculated + Win 5047	15	90
Inoculated + Vi-oxaline	5 ml.	100
Inoculated + Erythromycin	20	100

[1] Expressed as per cent dead bees heavily infected with Nosema after 24 days.

NOSEMA DISEASE

The efficacy of a number of substances* against Nosema disease has been tested (Table 2). The test procedure has been described elsewhere (5, 6) but, briefly, consisted of feeding these materials in 30 ml. 60 per cent sugar syrup containing about one million *Nosema apis* cysts per ml. to bees in wire cages (100 bees per cage), removing dead bees daily and examining for the presence of cysts in the epithelial cells of the ventriculus. The results presented are representative of those obtained in different experiments. It may be seen that Fumidil B is effective even at 5 mg. per 30 ml. solution. Since this material contains approximately 0.05 mg. pure fumagillin per mg. it is clear that 0.25 mg. of the pure compound was sufficient to control infection in 100 bees. This figure is close to that obtained in earlier trials reported by Katznelson and Jamieson (5). PA 106 or anisomycin reduced infection by about 50 per cent and was clearly much less effective than Fumidil B.

DISCUSSION

As the search for new antibiotics continues it is to be expected that others will be discovered which will be effective against AFB, Nosema and perhaps EFB as well. Achromycin, for example, appeared to be promising in controlling AFB; other substances such as erythromycin may also prove to be effective. Stability of these materials under hive conditions is one of the most important factors to be considered in this connection. Sulpha drugs are to be preferred, perhaps, to antibiotics such as terramycin by virtue of their greater stability, as well as their lower cost, in the treatment of AFB. However, a "dual-purpose" compound such as terramycin, which is effective against both AFB and EFB (6, 8), may be more useful despite its relative instability for it is evident that it is sufficiently stable to control AFB for a season (Table 1).

* Fumidil B is a fumagillin preparation from Abbott Laboratories; PA 106 is the antibiotic anisomycin supplied by Chas. Pfizer & Co. Inc.; Win 5047 is an amoebicidal agent produced by Sterling-Winthrop Research Institute, and Vi-oxaline is a 3.4 per cent solution of sodium sulphaquinozaline prepared by Vic Bin Limited. Erythromycin was obtained from the Lilly Laboratories.

Since the first announcement by Katznelson and Jamieson (5) of the ability of the antibiotic fumagillin to check Nosema, several reports have appeared which have substantiated these findings both in the laboratory and in the apiary (1, 2, 3). The impure concentrate Fumidil B also appears to be effective. There remain to be worked out suitable dosage levels and methods of application. Both Farrar (2) and Gochnauer (3) report success in the field with between 130 to 190 mg. fumagillin per gallon of syrup. On the basis of the data published elsewhere (4, 5), and confirmed in the present experiments (Table 2), considerably less material may be required, a factor which would render use of this antibiotic much less expensive. Further studies along these lines are in progress.

ACKNOWLEDGEMENTS

The authors are greatly indebted to the technical staff of the Apiculture Division, Experimental Farms Service, for manipulation of bees and colonies and for Nosema diagnosis. Grateful appreciation is also extended to the various firms mentioned above for supplying many of the chemicals used in this investigation.

REFERENCES

1. Bailey, L. Effect of fumagillin on *Nosema apis* (Zander). Nature 170 : 212-213. 1953.
2. Farrar, C. L. Fumagillin for Nosema control in package bees. Amer. Bee J. 94 : 52-53, 60. 1954.
3. Gochnauer, T. A. Chemical control of American foulbrood and Nosema diseases. Amer. Bee J. 93 : 410-411. 1953.
4. Jamieson, C. A., and H. Katznelson. Control of Nosema disease in colonies and its significance on honey production. Abstract in 15th International Bee Keeping Congress, Denmark. 1954.
5. Katznelson, H., and C. A. Jamieson. Control of Nosema disease of honeybees with fumagillin. Science 115 : 70-71. 1952.
6. Katznelson, H., and C. A. Jamieson. Antibiotics and other chemotherapeutic agents in the control of bee diseases. Sci. Agr. 32 : 219-225. 1952.
7. Katznelson, H., and C. A. Jamieson. Note on current studies on the chemotherapy of American foulbrood of the honeybee and on the stability of sulpha drugs in honey. Can. J. Agr. Sci. 34 : 120. 1954.
8. Katznelson, H., J. A. Arnott, and S. E. Bland. Preliminary report on the treatment of European foulbrood of honeybees with antibiotics. Sci. Agr. 32 : 180-184. 1952.

REACTION OF SMALL GRAINS TO VARIOUS DENSITIES OF WILD MUSTARD AND THE RESULTS OBTAINED AFTER THEIR REMOVAL WITH 2,4-D OR BY HAND

II. EXPERIMENTS WITH FLAX[1]

V. D. Burrows[2] and P. J. Olson[3]

[Received for publication December 28, 1954]

ABSTRACT

Flax (*Linum usitatissimum* L.), sown at the rate of 45 lb. per acre, was grown alone and in association with various densities of wild mustard (*Brassica arvensis* (L) Rabenh.). When the flax was 2-3 inches tall in 1952, and 1.0-1.5 inches tall in 1953, some of the plots were sprayed with 2,4-D butyl ester at the rate of 4 and 3 ounces acid equivalent per acre, respectively.

The experimental results demonstrated that: (*a*) spraying plots with a weed density of 10 or more mustard plants per square yard increased significantly the yield of grain, and (*b*) the presence of mustard in the plots, while the flax was in the seedling stage, affected adversely its basal branching, even when the weed was killed shortly afterwards with 2,4-D. The number of basal branches per flax plant was larger, however, in the sprayed plots than in the unsprayed plots. In 1952, the reduction of basal branching in the sprayed plots became more pronounced as the density of mustard was increased and resulted in a similar reduction in seed bolls per plant, weight of straw and yield of grain. In 1953, the flax in the sprayed plots apparently outgrew the effect of the early reduction in basal branching and produced yields of straw and grain approximately equal to the weed-free check.

INTRODUCTION

The first paper (3) of this series dealt with the reaction of wheat to various densities of wild mustard and the results obtained after their removal with 2,4-D or by hand. The present paper deals with the reaction of flax when subjected to similar conditions.

REVIEW OF LITERATURE

Robinson (10) studied the effect of annual weeds on the yield of wheat, oats and flax and found that complete removal of weeds by hand when the crop was 4 inches tall frequently resulted in an increase in yield. The increase raised the yield nearly to the level of that obtained from weed-free crops. Burrows and Olson (2) applied 2,4-D at the rate of 4 ounces acid equivalent per acre to flax plots infested with 81 weeds per square yard and obtained a yield increase of 12.66 bushels per acre.

It has been found that flax should be treated with 2,4-D or MCP as soon as weed growth warrants, provided the flax has reached a height of 2 inches (1). Flax in advanced stages of growth (from very early bud stage to about 10 days past full bloom) is susceptible to 2,4-D (7, 8), although some varieties seem to be more susceptible than others. Coupland (5) placed six varieties of flax in descending order of resistance to 2,4-D as follows: Redwing, Victory, Royal, Rocket, Dakota, and Arrow.

[1] Contribution from Division of Plant Science, The University of Manitoba, Winnipeg, Man.
[2] Research Assistant (now Graduate Assistant, Division of Biology, California Institute of Technology, Pasadena, Calif.)
[3] Professor of Plant Science (now retired, Grafton, North Dakota).

Chubb and MacKey (4) report that the oil content, iodine number, 1000-kernel weight, bushel weight and germination of seed from the varieties Liral Dominion, Liral Prince Toba and Royal flax were unaffected when the plants were sprayed with 2,4-D alkanolamine at rates of 2, 4, and 8 ounces acid equivalent per acre. Friesen (9) obtained no significant differences for oil content and iodine number between sprayed and unsprayed flax. On the other hand, Dunham (6) found that the yield of oil from some varieties was seriously reduced by even a 4-oz. application of both the sodium salt and ester formulations of 2,4-D, and that in some cases the iodine number of linseed oil was also reduced.

MATERIALS AND METHODS

This study was conducted at the University of Manitoba in the years 1952 and 1953. Flax was selected because of its tolerance at certain stages of growth to 2,4-D, and its relatively low ability to compete with weeds. The weed (wild mustard) was chosen because of its prevalence in Canada and its pronounced susceptibility to 2,4-D.

In 1952, the experiment consisted of quadruplicate blocks, each of which contained seven randomized main plots with different densities (0, 10,-25, 50, 100, 200, and 400 plants per square yard) of wild mustard. The density of flax plants (variety Dakota sown at 45 lb. per acre) was, however, uniform for all plots. Each main plot was divided into two sub-plots, one of which was sprayed with 2,4-D and the other left unsprayed.

In 1953, the experiment also consisted of quadruplicate blocks, but the range of mustard density in the main plots was reduced (0, 5, 10, 25, 50, and 100 plants per square yard), and the variety Redwood (sown at 45 lb. per acre) was substituted for Dakota. Each main plot was subdivided into unsprayed, sprayed, and hand-weeded sub-plots.

The methods used to establish the stands of wild mustard in flax were the same as those previously described (3) for wheat.

The flax was sprayed with 2,4-D butyl ester at the rate of 4 ounces acid equivalent per acre in 1952, and at the rate of 3 ounces per acre in 1953. In 1952, the flax at the time of spraying was 2 to 3 inches tall (plants in true leaf stage) and the mustard 3 to 4 inches tall, while, in 1953, 2,4-D was applied when the flax and the mustard both were 1 to $1\frac{1}{2}$ inches in height. In 1953, the hand-weeding and spraying were done on the same day.

When the flax was mature, the plots were trimmed to eliminate any 'border' effect. In 1952, the trimmed plots were 16.5 feet in length and six drill rows in width, but in 1953 they were 10 feet in length and four drill rows in width. The data on the number of plants per plot, basal branches per plant, seed bolls per plant, and the total yield of flax and weight of mustard (unsprayed plots) were obtained after harvest, and those regarding the weight of straw (sheaf weight minus grain weight), the yield of grain, the bushel and 1000-kernel weight, the commercial grade of the seed, and the iodine number of the oil were obtained after threshing. The mean number of bolls per plant was determined from counts on 25 plants in 1952, and 50 plants in 1953, the plants being taken from comparable locations in each plot.

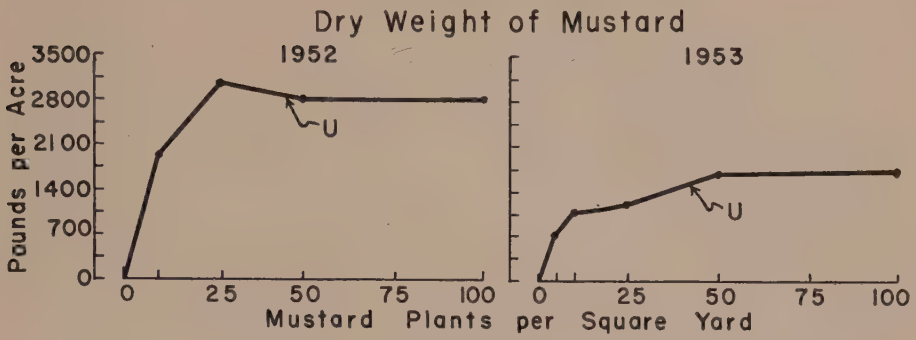

FIGURE 1. Dry weight of wild mustard (pounds per acre), at various levels of density in unsprayed (U) plots of flax sown at the rate of 45 lb. per acre.

EXPERIMENTAL RESULTS

Dry Weight of Wild Mustard

The trend for weights of mustard for the unsprayed plots, at harvest time, is shown in Figure 1. In Figure 1 and subsequent figures, the 1952 means for the 200 and 400 mustard plant densities are not included because they are essentially the same as the 100 mustard plant density. Although observations in the field showed that the growth of individual mustard plants became less as their number per square yard increased, the data obtained show that, beyond the 25 plant per square yard level in 1952, and the 50 plant level in 1953, the total dry weight of the mustard remained about the same. At and below these levels, the mustard plants tended to grow very large, as the flax in association with them offered very little competition. However, as the density of the weed increased, the competition between the mustard plants themselves increased, resulting in a nearly constant dry weight of mustard per plot. The weeds below the 25 density level in 1952, and the 50 level in 1953, were very vigorous and grew very tall but, because of their reduced numbers, their total dry weight was less.

TABLE 1.—NUMBER OF FLAX PLANTS IN UNSPRAYED, SPRAYED AND HAND-WEEDED PLOTS AT VARIOUS LEVELS OF MUSTARD PLANT DENSITY

Treatment	Density of wild mustard						
	0	5	10	25	50	100	Mean[2]
Unsprayed	836.7	912.2	861.0	791.0	447.7	347.2	698.8
Sprayed	839.0	860.0	984.5	928.0	825.5	863.2	883.3
Hand-weeded	898.2	855.5	889.0	762.2	816.0	910.0	855.1
Mean[1]	857.9	875.9	911.5	827.1	695.4	706.8	

[1] Least significant difference (5 per cent level) between means for different levels of mustard density = 171.1 plants; and for different levels of mustard density for each treatment = 433.6 plants.

[2] Least significant difference (5 per cent level) between means for treatments = 88.6 plants; of treatments at each level of wild mustard density = 217.0 plants; and of interaction (treatment × level of wild mustard density) = 194.1 plants.

Flax Plants Per Plot

Observations made during the growing season of 1952 indicated that wild mustard tended to thin the stand of flax through competition. In general, there was a decrease in the average number of flax plants per plot with increase in the level of weed density, but the differences between levels were not significant. Spraying the plots with 2,4-D did not increase significantly the average number of plants. In 1953 (Table 1), many of the flax plants in the unsprayed plots at the 50 and 100 weed density levels were killed during the growing season by wild mustard. The average number of flax plants in these unsprayed plots were significantly lower than those which were sprayed or hand-weeded.

Basal Branches Per Plant

The average counts of basal branches per plant are illustrated in Figure 2. In both years, 1952 and 1953, the presence of wild mustard in the unsprayed plots reduced basal branching of the flax plants. Branching was least at the 25 mustard plant per square yard density in 1952, and at the 100 plant density in 1953. The removal of weeds with 2,4-D permitted more branching in both years, but the number of branches was less than where there were originally no mustard plants and it decreased as the density of wild mustard increased. Apparently, the presence of wild mustard for even the short interval between the time the flax emerged and the time the weed was *completely destroyed* by 2,4-D was sufficient to reduce basal branching. In the hand-weeded plots the average number of basal branches was about the same as the weed-free plots. This is taken as evidence that the competition from the weed up to the date of spraying was not strong enough to reduce basal branching. It must be pointed out that the duration of weed competition was greater in the sprayed plots than in the hand-weeded plots. In the sprayed plots it took several days after the time of spraying for the wild mustard to die, whereas in the hand-weeded plots competition ceased as soon as the plants were removed.

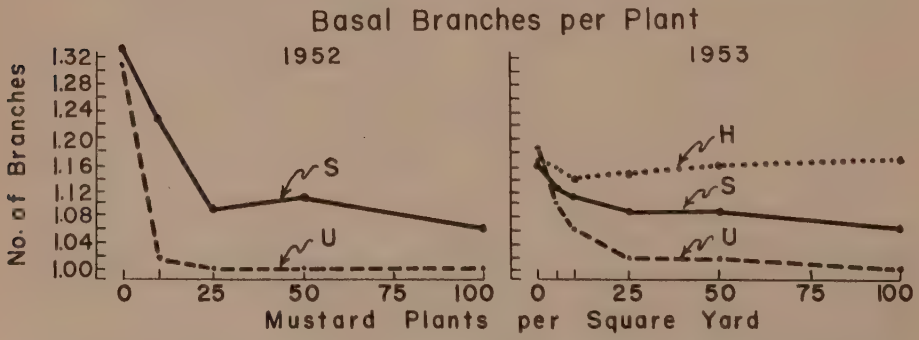

FIGURE 2. Basal branches per plant in unsprayed (U), sprayed (S) and hand-weeded (H) plots of flax sown at the rate of 45 lb. per acre and grown in association with various densities of wild mustard.

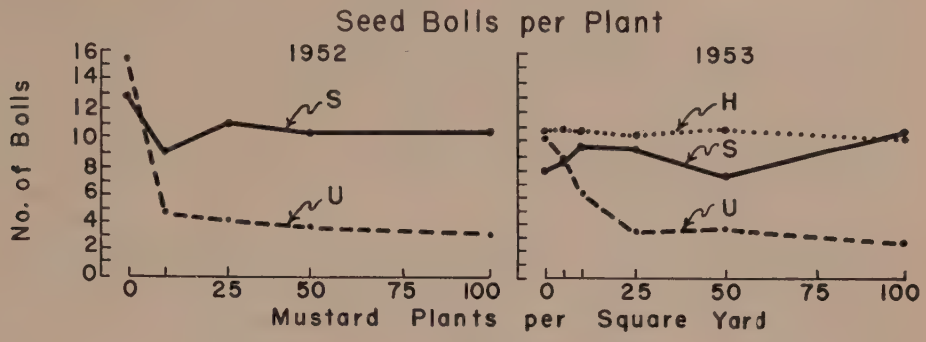

FIGURE 3. Seed bolls per plant in unsprayed (U), sprayed (S) and hand-weeded (H) plots of flax sown at the rate of 45 lb. per acre and grown in association with various densities of wild mustard.

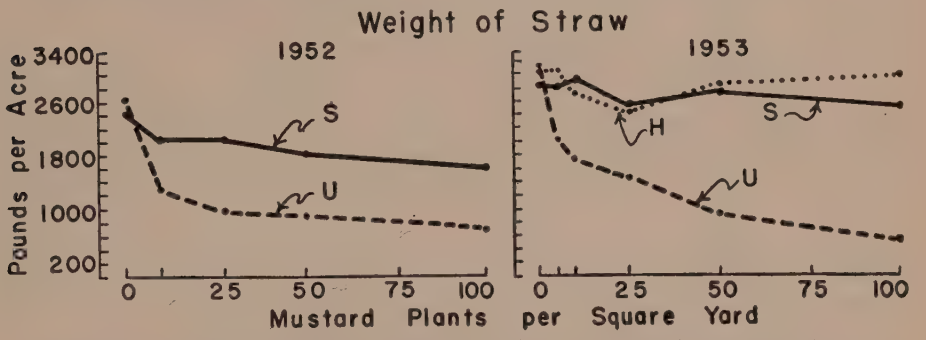

FIGURE 4. Weight of straw (pounds per acre) in unsprayed (U), sprayed (S) and hand-weeded (H) plots of flax sown at the rate of 45 lb. per acre and grown in association with various densities of wild mustard.

Seed Bolls Per Plant

Observations of weedy and weed-free plots showed that, in both years, there was a striking difference in the production of seed bolls (Figure 3). In the unsprayed plots of 1952 the presence of only 10 weeds per square yard reduced boll production to the minimum but, in 1953, 25 weeds per square yard were required. Weedy plots sprayed with 2,4-D produced more seed bolls per plant, but spraying weed-free plots resulted in a slight decrease which was not significant.

In 1953, there was no significant difference between the boll count of the sprayed and hand-weeded plots or between the different levels of mustard density. Apparently, in this instance, the early reduction in basal branching in the sprayed plots was compensated for by a greatly increased top branching.

Weight of Straw

Since the growth of flax was known to be reduced by weeds, it seemed desirable to obtain the yield of flax straw for each plot. Figure 4 shows that competition from wild mustard in densities up to the 100 population level markedly reduced the yields of flax straw in the unsprayed plots in both years and also, to a smaller amount, in the sprayed plots of the 1952 trial. The gradient of decrease was quite steep at low weed densities but

it tended to level off at higher densities. The 1952 results reveal also the effects of early competition from wild mustard, for where mustard was initially present there was less straw than where there was initially no mustard. For the sprayed and unsprayed weed-free plots the difference is not significant but the lower yield of the sprayed plots may indicate slight injury from the spray.

The sprayed and hand-weeded plots were not significantly different from each other with respect to the yield of straw. This is further evidence that the flax in the sprayed plots had overcome the early handicap from the reduction in basal branching when the plants were young.

Yield of Grain

The trend in the yield for linseed under different conditions is illustrated in Figure 5. In both years, competition from wild mustard in the unsprayed plots severely reduced the yields of linseed.

In 1952, a density of only 10 mustard plants per square yard was sufficient to reduce sharply the yield of flax. Additional increase in weed density reduced the yield further but the gradient of the decline was much less steep. The sprayed plots of 1952 also showed a progressive decrease in yield, but the reduction at the 10 mustard plant level was much less marked than in the unsprayed plots. It is noteworthy that the reduction already referred to, in basal branching early in the growing season, resulted in a reduction in the yield of grain as well as of straw.

In 1953, the yield of grain from the unsprayed plots decreased progressively as the density of mustard increased. With only one exception (the 10 mustard plant level) the yield from sprayed plots was not significantly different from the yield of the hand-weeded plots at any of the mustard density levels studied.

In both years, the difference between the yields of the sprayed and the unsprayed flax plots was very pronounced and showed that the gain from weed control could be very large.

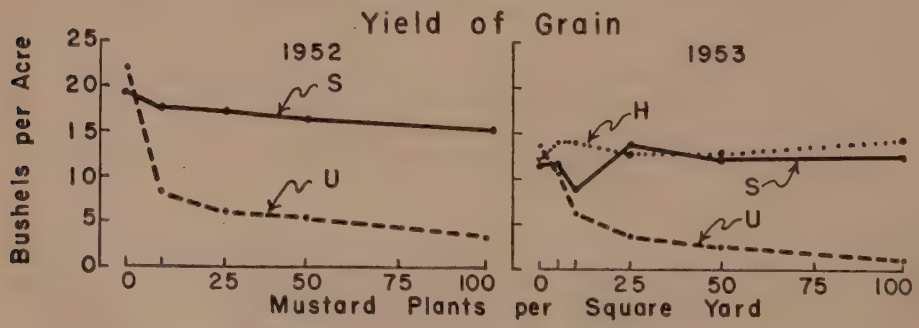

FIGURE 5. Yield of grain (bushels per acre) in unsprayed (U), sprayed (S) and hand-weeded (H) plots of flax sown at the rate of 45 lb. per acre and grown in association with various densities of wild mustard.

TABLE 2.—BUSHEL WEIGHT OF LINSEED FROM UNSPRAYED, SPRAYED AND HAND-WEEDED PLOTS AT VARIOUS LEVELS OF MUSTARD DENSITY

Treatment	Density of wild mustard						
	0	5	10	25	50	100	Mean[2]
Unsprayed	57.1	55.7	56.6	56.2	56.5	53.5	55.9
Sprayed	56.3	56.4	56.7	56.3	56.6	56.7	56.5
Hand-weeded	56.8	56.9	56.7	56.5	56.4	57.2	56.8
Mean[1]	56.7	56.3	56.7	56.3	56.5	55.8	

[1] Difference between means for different densities of wild mustard were not significant.
[2] Least significant difference (5 per cent level) between means for treatments = 0.87 lb.; and of treatments at each level of wild mustard density = 2.14 lb.

TABLE 3.—1000-KERNEL WEIGHT (GRAMS) OF LINSEED FROM SPRAYED AND UNSPRAYED PLOTS AT VARIOUS LEVELS OF MUSTARD PLANT DENSITY

Treatment	Density of wild mustard							
	0	10	25	50	100	200	400	Mean[2]
Sprayed	5.71	5.82	5.76	5.56	5.73	5.85	5.76	5.74
Unsprayed	5.72	6.04	6.01	6.12	6.08	6.16	6.00	6.02
Mean[1]	5.71	5.93	5.88	5.84	5.90	6.00	5.88	

[1] Differences between means for different densities of wild mustard were not significant.
[2] Least significant difference (5 per cent level) between means for treatments = 0.18 grams.

Bushel Weight and 1000-Kernel Weight

In 1952, the bushel weight of the seed was not affected by either a variation in the density of wild mustard or by spraying with 2,4-D. In 1953, the average bushel weight of the unsprayed plots was significantly lower than that of the hand-weeded plots, but not of the sprayed plots. There was no differential response to weed density. The results obtained are shown in Table 2.

In 1952, the 1000-kernel weight was significantly higher in the unsprayed plots than in the sprayed, but the differences between the weights for the unsprayed plots at different levels of mustard density were not significant. The results obtained are shown in Table 3. In 1953, the differences resulting from variation in plot treatment or mustard density were not significant.

Commercial Grade, Oil Content and Iodine Number

The commercial grade and oil content of linseed in both years, and the iodine number of the oil in 1952, were unaffected by either 2,4-D or by differences in mustard density. In 1953, however, weeds in the unsprayed plots significantly reduced the iodine number of the oil. The data on iodine number for 1953 are summarized in Table 4.

TABLE 4.—IODINE NUMBER OF LINSEED OIL IN UNSPRAYED, SPRAYED AND HAND-WEEDED PLOTS AT VARIOUS LEVELS OF MUSTARD PLANT DENSITY

Treatment	Density of wild mustard						
	0	5	10	25	50	100	Mean[1]
Unsprayed	188.2	185.3	185.3	183.9	181.6	180.7	184.2
Sprayed	186.6	186.5	186.0	189.4	186.3	187.7	187.1
Hand-weeded	186.7	188.3	189.1	190.0	186.7	189.1	188.3

[1] Least significant difference (5 per cent level) between means for treatments = 2.5.

DISCUSSION AND CONCLUSIONS

In both years, it was found that the presence of wild mustard in plots of flax caused a serious reduction in its vigour and growth. In 1953, it was found that mustard also reduced the stand of flax at the 50 and 100 mustard plant per square yard density levels. Further effects were a reduction of basal branching in young flax plants and a consequent reduction in the number of seed bolls per plant, weight of straw and yield of grain.

In both years, the removal of wild mustard with 2,4-D resulted in significant increases in basal branches per plant, seed bolls per plant, weight of straw, and yield of grain. The gains in basal branching in both years, and the gain in seed bolls per plant, weight of straw, and yield of grain in 1952, did not, however, reach the level found in weed-free plots. Since the crops were treated at an early stage of growth, the adverse effect of wild mustard on basal branching of flax was unexpected. This result, therefore, demonstrates that competition from weeds during the early stage of flax growth is important and it suggests that spraying of flax with 2,4-D should be done as early in the growing season as possible to shorten the duration of weed competition, but not until after the cotyledon stage of growth.

In sprayed plots, evidence has already been presented (1953 results) to show that, in flax, the early reduction in basal branching may be compensated for by a greatly increased top branching. This may have been due to the higher soil moisture in 1953—16.33 inches of total precipitation, compared to 10.53 inches of total precipitation in 1952.

Evidence is presented to show that flax is unable to compete with even a relatively small infestation of wild mustard, and that the removal of a mustard density of 10 plants per square yard with 2,4-D is fully justified.

ACKNOWLEDGEMENTS

Grateful acknowledgement is extended to: Canadian Industries Limited for providing the financial assistance which made this study possible; and to J. A. Anderson, Chief Chemist, Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, for making the chemical determinations reported in this paper.

REFERENCES

1. Anonymous. Report of the herbicide and weed classification committee. Proc. of the Western Canadian Weed Control Conf. 7 : 171-180. 1953.
2. Burrows, V. D., and P. J. Olson. Effect of chemical control on yield of flax. Proc. and Abstracts of the Western Canadian Weed Control Conf. 5 : 39-40. 1951.
3. Burrows, V. D., and P. J. Olson. Reaction of small grains to various densities of wild mustard and the results obtained after their removal with 2,4-D or by hand. I. Experiments with wheat. Can. J. Agr. Sci. 35 : 68-75. 1955.
4. Chubb, W. O., and E. M. MacKey. Effect of 2,4-D sprays on fiber flax. Res. Rept. North Central Weed Control Conf. 6 : 81. 1949.
5. Coupland, R. T. Differential response of six varieties of flax to 2,4-D. Proc. and Abstracts of the Western Canadian Weed Control Conf. 5 : 41. 1951.
6. Dunham, R. S. Response of Koto flax to application of 2,4-D. Proc. North Central Weed Control Conf. 4 : 35-36. 1947.
7. Foster, J. R. The effect of 2,4-D on flax when applied at different growth stages. Res. Rept. North Central Weed Control Conf. 6 : 84. 1949.
8. Friesen, G., and P. J. Olson. Reaction of flax to 2,4-D applied at different stages of growth. Proc. and Abstracts of the Western Canadian Weed Control Conf. 5 : 41-42. 1951.
9. Friesen, H. A. Effect of 2,4-D on the quality of spring wheat and linseed flax. Res. Rept. North Central Weed Control Conf. 6 : 85. 1949.
10. Robinson, R. G. Annual weeds, their viable seed population in the soil, and their effect on yields of oats, wheat and flax. Agron. J. 41 : 513-518. 1949.

EFFECT OF BACTERIAL WILT ON HAY YIELD OF IRRIGATED ALFALFA[1]

R. W. Peake[2] and M. W. Cormack[3]

Experimental Station and Science Service Laboratories, Lethbridge, Alberta

[Received for publication December 3, 1954]

ABSTRACT

Bacterial wilt markedly reduced the hay yield of Grimm alfalfa in the rotation plots at Lethbridge after 1940. The average yield reduction from the fourth to the sixth crop year was 57.7 per cent as compared to 8.7 per cent in the corresponding period before the disease became established. Although yields were generally higher in the fertilized than in the unfertilized plots, relatively the same losses were caused by bacterial wilt. A disease analysis showed that infection developed gradually until the third crop year, after which there was a rapid increase.

In a 6-year study of 10 alfalfa varieties, the hay yields of Grimm, Canauto, Ferax, and Rhizoma were greatly reduced after the third crop year, and those of Ladak, Buffalo, Cossack, and Viking dropped off rapidly during the fifth and sixth year. The wilt-resistant varieties, Ranger and Hardistan, produced good yields during the entire period. In another experiment, where conditions were particularly favourable for disease development, all varieties tested, except Hardistan, Orestan, and Wisconsin Synthetic C, were seriously reduced in stand and yield between the first and second crop year. Disease ratings showed that this damage was caused by bacterial wilt and in the case of Buffalo and Ranger, winter injury contributed to the stand reductions observed.

INTRODUCTION

The successful production of alfalfa is of prime importance to the irrigation farmer in southern Alberta and many other areas. This crop is an essential part of nearly all rotations, and serious losses may result if it fails to produce satisfactory yields. For this reason the spread of bacterial wilt into southern Alberta about 1939 (1) was the cause of considerable concern. As in many areas in the United States (5), this disease soon became widespread and greatly reduced the yields and shortened the life of irrigated stands of the commonly grown variety Grimm (2).

The effects of bacterial wilt on alfalfa have been extensively studied in the United States (3, 4, 5, 9, 10). Usually disease development has been measured by determining the percentage stand or the degree of infection of the plants, and relatively little attention has been paid to hay yield. Field plot yields, however, were successfully used in comparing the performance of alfalfa varieties in wilt infested soil in Colorado (10). The primary purpose of the present study was to obtain accurate information on the effect of the disease on alfalfa hay yield in the irrigated areas of southern Alberta.

For many years, alfalfa has been under study at the Lethbridge Experimental Station. These investigations have included long- and short-term rotations, fertility experiments, and variety tests (7). Because of the diversity of these tests, it was possible to include several factors in studies on

[1] Joint contribution from the Forage Crops Division, Experimental Farms Service, and the Botany and Plant Pathology Division, Science Service—Contribution No. 1423—Canada Department of Agriculture.
[2] Head, Forage Crops Section.
[3] Officer-in-Charge, Botany and Plant Pathology Laboratory.

the effect of bacterial wilt on irrigated alfalfa. Hay yields were obtained on rotation stands, 1 to 6 years old, for several years before and after the advent of bacterial wilt. Disease ratings were made on stands of varying age and of different varieties. This report also includes hay yield data on ten varieties for a 6-year period, and on another varietal test during two years when conditions were particularly favourable for development of wilt.

STUDIES ON ROTATION PLOTS

One of the major rotations under irrigation at Lethbridge was established in 1910 on a 10-year basis with wheat, alfalfa-6 years, oats, barley, and a cultivated crop. Originally potatoes were the cultivated crop, but in 1923 they were replaced by sugar beets which were continued during the period of this study. The plots were one acre each. Twice during the 10-year rotation 15 tons of barnyard manure were applied to each plot. In addition, one-half of each plot received 100 pounds of fertilizer (ammonium phosphate, 11-48) when beets were planted, and during the first and fourth year of the alfalfa. With this rotational arrangement it was possible to study the effect of bacterial wilt on Grimm alfalfa from 1 to 6 years old at two fertility levels.

Hay Yields

The average yields of alfalfa hay from the rotation stands 1 to 6 years old before and after the invasion of bacterial wilt are shown in Table 1. The period 1936 to 1940 was the 5 years immediately before the disease had any noticeable effect, and the period 1941 to 1945 included the first 5 years in which it was becoming a serious threat to alfalfa production in southern Alberta.

Previous to the invasion of bacterial wilt, yields of alfalfa in the 10-year rotation stayed at a fairly constant level from the second to the sixth year, with the first-year yields generally about two-thirds of those of the following year. After bacterial wilt became a factor (1941-45), yield reductions were recorded in the fourth year, and they became marked in the fifth and sixth years.

TABLE 1.—AVERAGE YIELD OF ALFALFA HAY FROM FERTILIZED AND UNFERTILIZED STANDS 1 TO 6 YEARS OLD, BEFORE AND FOLLOWING THE INVASION OF BACTERIAL WILT

Age of stand	Tons per acre, 12 per cent moisture			
	Fertilized		Unfertilized	
Years	1936-40	1941-45	1936-40	1941-45
1	2.49	1.95	1.94	1.82
2	4.37	3.92	2.74	1.84
3	3.82	4.32	2.75	2.63
4	4.25	3.98	2.44	2.54
5	3.59	2.49	1.89	1.40
6	3.41	1.56	2.69	1.20

Previous to 1940, the average yield reduction from the fourth to the sixth year on fertilized fields was 19.8 per cent. After 1940, yields from the fourth to the sixth year were reduced 60.8 per cent. The data show that fertilizer generally increased yields throughout the experiments, but had no effect on the yield reductions caused by bacterial wilt after 1940. Before bacterial wilt became a factor, alfalfa yields on unfertilized plots remained high until the sixth year, with an actual increase of 10.2 per cent between the fourth and sixth year. After 1940, the yield reduction between the fourth and sixth year was 52.8 per cent.

Disease Development

The alfalfa plots 1 to 6 years old were sampled and rated for bacterial wilt in the fall of 1945 after final hay yield data had been obtained for the period 1941 to 1945. Ten randomized quarter metre quadrat samples were obtained in each plot. The average number of plants in each sample varied from 50 in the young stands to 10 in the thin, 6-year-old stand, in which many plants had been previously killed. All plants were rated for bacterial wilt infection on the basis of top and root symptoms, using a numerical rating of 0. to 5. The average percentages of diseased plants with varying degrees of infection were determined for stands of each age (Table 2).

TABLE 2.—DEVELOPMENT OF BACTERIAL WILT IN ROTATION PLOTS OF GRIMM ALFALFA 1 TO 6 YEARS OLD

Age of stand	Percentage of diseased plants			
	Root symptoms only	Early top symptoms	Dead or dying	Total
1 year	1.2	—	—	1.2
2 years	0.9	—	—	0.9
3 years	20.8	0.5	—	21.3
4 years	22.2	11.3	12.1	45.6
5 years	19.4	14.8	28.8	63.0
6 years	15.3	10.5	63.8	89.6

The early stages of infection in the 1- and 2-year-old stands were detectable only in the roots. In the 3-year-old stand over 20 per cent of the plants were infected, with a few showing top symptoms. The disease developed very rapidly in the older stands until nearly 90 per cent of the plants were infected in the sixth year. Thinning of the stand through death of the plants appeared in the fourth year plot in which 12.1 per cent of the plants were dead or dying. The increase of this damage to 63.8 per cent in the sixth year explains the marked reductions in hay yield in the older stands (Table 1).

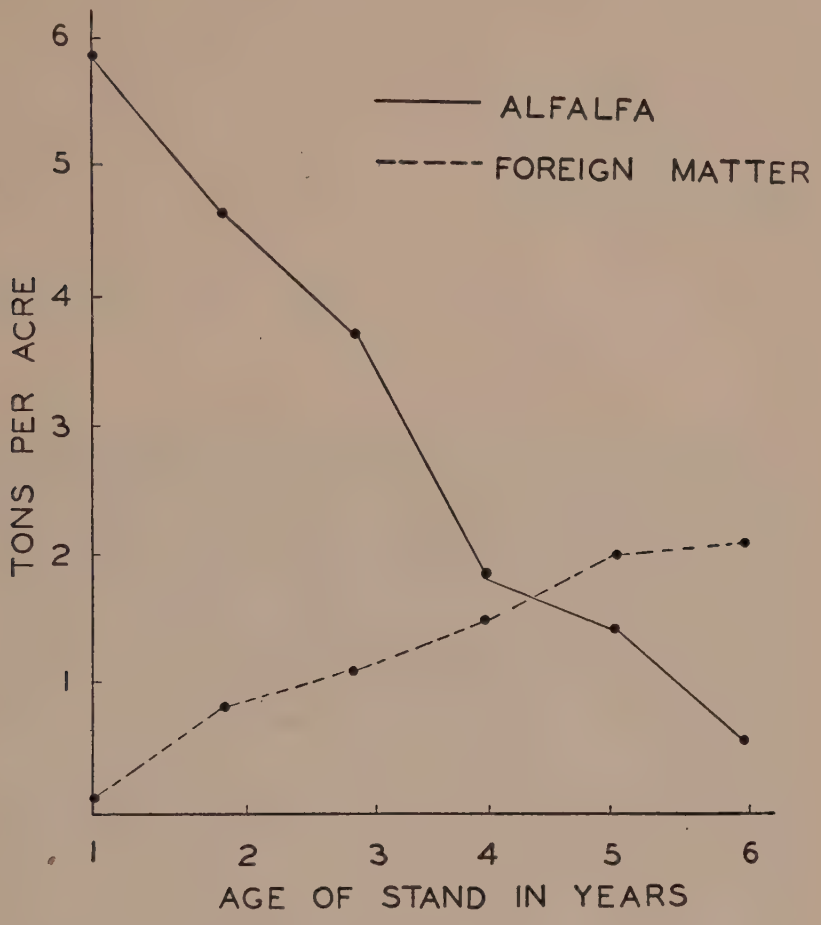

FIGURE 1. Reduction of yield of Grimm alfalfa caused by bacterial wilt and encroachment of foreign growth.

STUDIES ON ALFALFA VARIETIES

Hay Yields

Yield data have been collected from two sets of variety tests. In the first test, started in May, 1945, 10 varieties of alfalfa were seeded in plots 15 × 20 feet in a randomized block of four replicates each. The second test, seeded in May 1950, involved 10 varieties replicated four times in plots 10 × 20 feet. Water was applied as required, usually by flood irrigation, three times or occasionally four, during each season.

Harvesting procedure consisted of mowing a strip, 3 feet wide and of a recorded length of approximately 16 feet, from the centre of each plot. Green weights were taken in the field, using a Toledo hanging scale accurate to one ounce. Dry matter samples were taken from each plot. Because weeds and foreign growth invade alfalfa fields as bacterial wilt reduces stand, a botanical separation was made for each plot clipped. Yields are

reported in tons of hay per acre at 12 per cent moisture of pure alfalfa. Notes were also taken on spring recovery, winter damage, and recovery after cutting. The varieties tested are listed in Table 3 and Figure 4.

Yields of hay in the first test covering a 6-year period are presented in Table 3. The yields of Grimm, Canauto, Ferax, and Rhizoma started to decline after the second crop year, were greatly reduced between the third and fourth years, and continued to go down rapidly. The marked reduction in Grimm is illustrated in Figure 1. Yield reductions were not apparent in the other varieties until between the third and fourth years. Yields of Ladak, Buffalo, Cossack, and Viking dropped off rather rapidly during the fifth and sixth years, but Ranger and Hardistan contined good production throughout the experiment (Figures 2 and 3).

The reduction of the alfalfa stand by bacterial wilt is accompanied by an invasion by weeds and grasses. In the early years, milkweed and dandelion appear first, followed by brome grass and Kentucky bluegrass. Botanical separations made at the time of harvest were used to determine the amount of foreign growth. An indication of the amount of foreign growth is presented in Figure 1.

Severe winter-injury occurred in two varieties, causing a considerable reduction in stand in Buffalo and some damage in Ranger. For example, following the winter of 1946-47, the estimated injury was Buffalo, 28 per cent, and Ranger, 14 per cent, while Ladak was 7 per cent, and Grimm 2 per cent. All other yield and stand reductions were attributed to bacterial wilt since no other serious diseases were observed.

TABLE 3.—HAY FIELD OF TEN VARIETIES OF ALFALFA FOR SIX YEARS UNDER IRRIGATION

Tons per acre at 12 per cent moisture

Variety	1946	1947	1948	1949	1950	1951	6-year average
Hardistan	5.16	4.34	5.01	4.48	4.64	3.48	4.51
Ladak	5.97	4.80	5.35	4.12	3.83	2.32	4.40
Ranger	5.39	4.60	5.05	4.39	3.92	3.06	4.40
Cossack	5.27	4.86	5.47	4.42	3.60	2.49	4.36
Viking	5.60	4.48	4.87	3.91	3.08	1.43	3.89
Buffalo	4.86	4.08	4.78	3.76	3.46	2.30	3.87
Rhizoma	5.67	4.30	3.73	2.37	1.43	0.68	3.03
Grimm	5.85	4.64	3.72	1.83	1.42	0.59	3.00
Ferax	5.34	4.47	3.14	1.18	0.96	0.42	2.58
Canauto	5.06	4.13	2.66	0.58	0.34	0.20	2.16

5 per cent L.S.D.	0.478
1 per cent L.S.D.	0.645

FIGURE 2. General view of alfalfa plots showing how some varieties were destroyed by bacterial wilt.

FIGURE 3. Comparison of alfalfa varieties resistant and susceptible to bacterial wilt. At the end of four years Hardistan (*top*) retained a perfect stand while Rhizoma (*bottom*) was almost completely destroyed.

Invasion of an alfalfa field by bacterial wilt is usually a gradual process and yield reductions are seldom marked before the fourth year. However, under conditions that are particularly favourable for the advance of the disease, stand reductions have occurred much earlier and in a much more severe form. This was evidenced in the second variety test seeded in 1950. Excellent stands of all varieties were obtained and a light clipping was taken from the experimental plots during the year of seeding. The first year yields taken in 1951 were average. Cool, moist weather during the 1951 and 1952 seasons was favourable for the early development of bacterial wilt. These conditions coupled with the close proximity of the disease in adjoining plots brought about a rapid spread of infection that caused very marked stand and yield reductions between the first and second crop years (Figure 4). Only three varieties, Hardistan, Wisconsin Synthetic C., and Orestan, showed little or no reduction in the second year. All of the other varieties showed a very marked reduction. Grimm was the highest-yielding variety in the first year and the lowest-yielding in the second year.

At the end of the second year the percentage stand on these plots was determined by a point quadrat method. In using this method a number of points fell between the drill rows of alfalfa so that an approximately 70 per cent stand is recorded in a perfect stand of alfalfa. The stand for Wisconsin Synthetic C and Ranger was 70 per cent; Hardistan and Orestan 65 per cent; and Buffalo 60 per cent. All others were greatly reduced. The stand survival for Cossack was 48 per cent; Ladak 43 per cent; Viking 38 per cent; Hardigan 30 per cent; and Grimm 23 per cent.

Disease Development

Plots of seven varieties of alfalfa seeded on irrigated land at Lethbridge in 1940 were sampled and rated for bacterial wilt development in 1945, using the method described for the rotation study. In the late fall of 1945 the plots were ploughed, and the roots of all remaining plants of each variety were examined for bacterial wilt.

TABLE 4.—DEVELOPMENT OF BACTERIAL WILT IN 5-YEAR-OLD PLOTS OF SEVEN VARIETIES OF ALFALFA

Variety	Root symptoms only	Sample analysis		Mass root study	
		Top symptoms or death	Total diseased	Number examined	Diseased
	%	%	%		%
Ranger	23.7	4.2	27.9	463	20.7
Ladak	25.1	9.2	34.3	998	37.3
Viking	29.6	4.5	34.1	850	42.3
Canauto	33.7	24.5	58.2	669	64.3
Grimm	43.6	30.4	74.0	1027	82.9
Ont. Variegated	41.4	42.0	83.4	833	62.9
Rhizoma	46.1	45.3	91.4	585	86.7

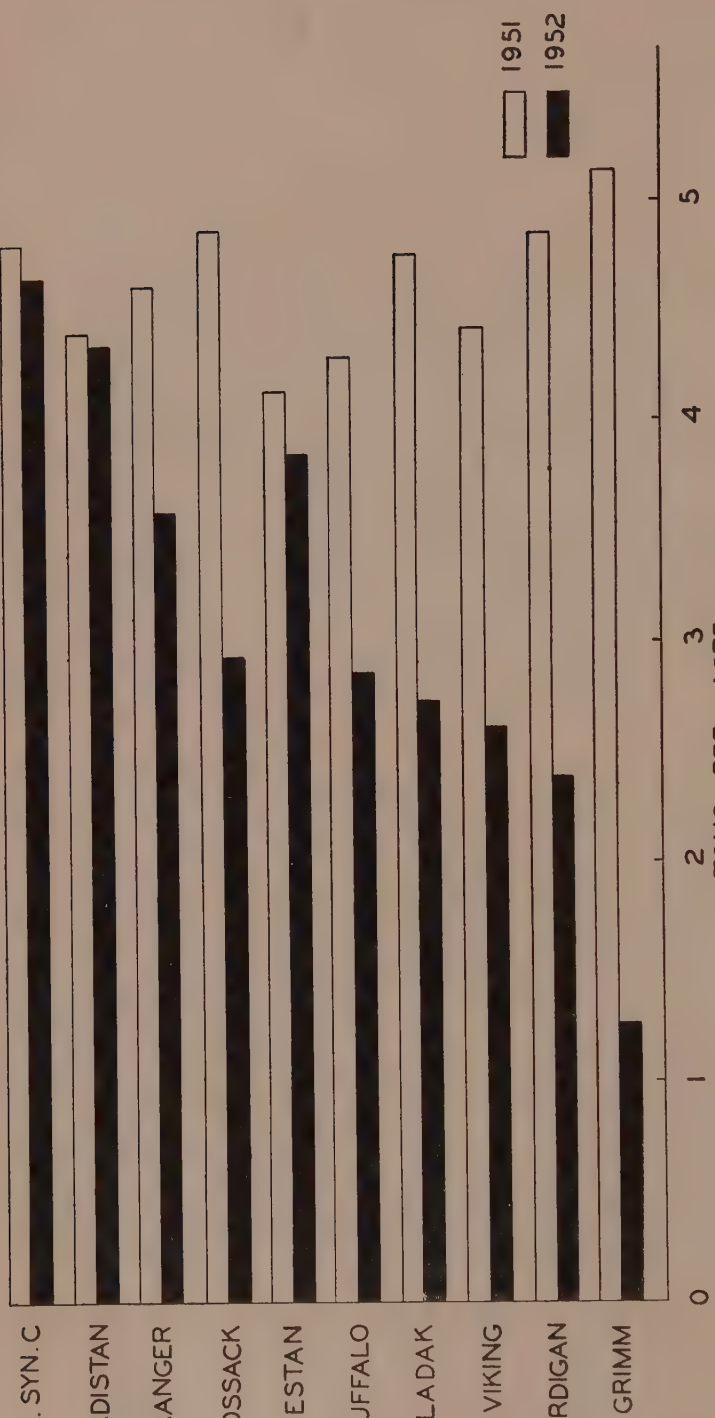

FIGURE 4. Reduction in yield caused by bacterial wilt between the first and second crop year in irrigation stands of alfalfa varieties.

As shown in Table 4, the varieties differed greatly in reaction to the disease. The results of the sampling method and the mass root examination were in general agreement. The variety Ranger contained the lowest percentage of diseased plants, but Ladak and Viking also showed considerable resistance. Canauto, Ontario Variegated, Grimm, and Rhizoma were high in percentage of total infection and in plants with top symptoms.

These results were confirmed in observational plots at Brooks and Lethbridge, in which additional varieties were included. At both locations, the susceptible varieties contained many infected plants within two years and were severely damaged by the end of the fourth year. Other varieties still had a good stand. On this basis the varieties studied were grouped as follows: *Highly resistant* (85-90 per cent survival)—Hardistan, Orestan, Ranger, and Wisconsin wilt-resistant strains; *Moderately resistant* (50-60 per cent survival)—Cossack, Ladak, and Viking; *Highly susceptible* (10-25 per cent survival)—Canauto, Ferax, Grimm, Ontario Variegated, and Rhizoma.

In all experiments the highly susceptible varieties were those that declined most rapidly in hay yield after the second year (Table 3). Buffalo was the only variety that suffered appreciably from winter injury in these tests.

DISCUSSION

The results of this study emphasize the importance of bacterial wilt as a major limiting factor in the production of irrigated alfalfa. Before this disease became established in southern Alberta, the commonly grown variety Grimm was reasonably satisfactory in hardiness and yield, and alfalfa stands could be maintained for many years. This situation soon changed when the disease became widespread after 1940. As indicated by the hay yield data presented, Grimm stands were seriously reduced in yield and their productive life was shortened by one-half or more. In the irrigation areas, this variety can now be profitably grown only in short rotations. These results stress the need for a wilt-resistant variety.

The data from the variety tests were in general agreement with those reported from other areas (3, 4, 6, 9, 10). Of the previously untested varieties of Canadian origin, Canauto, Ferax, and Rhizoma proved equally susceptible to Grimm. The variety Viking, however, ranked with Ladak and Cossack as moderately resistant. All of the highly resistant varieties tested were developed in the United States as the result of many years of selection and breeding. In early studies (3, 4, 9), it was shown that Turkistan and Ladak, also of Asiatic origin, were the best sources of resistance. The wilt-resistant varieties Hardistan and Orestan, developed from Turkistan, have never been widely distributed. The more recently developed varieties Ranger and Buffalo are now extensively grown in parts of the United States. The new variety, Vernal, developed in Wisconsin, was not available for this study but Wisconsin Synthetic C, a strain of similar origin, proved highly resistant.

All alfalfa varieties highly resistant to bacterial wilt that are now available appear to have definite limitations in Western Canada. Hardistan has proved highly susceptible to leaf diseases (5). Ranger and Buffalo are,

unfortunately, not dependably winter hardy (8). The new variety Vernal shows promise but it has not been thoroughly tested for adaptation and resistance to other diseases in Western Canada. In the meantime, Ladak is being recommended as a stop-gap variety in southern Alberta since it possesses a certain degree of wilt resistance in addition to other desirable qualities (6, 8). Seed of Ladak is available in reasonable quantities. However, Ladak is variable in performance (5), and it will not withstand severe disease conditions such as occurred in the second varietal test. An extensive selection and breeding program is in progress in an attempt to produce a variety combining high disease resistance with satisfactory hardiness and yield.

REFERENCES

1. Cormack, M. W. Bacterial wilt. *In* 19th Annual Report of the Canadian Plant Disease Survey, 1939, pp. 24-25. 1940.
2. Cormack, M. W. Bacterial wilt of alfalfa. Can. Dept. Agr. multilithed circ., 4 pp. 1943.
3. Graber, L. F., and F. R. Jones. Varietal survival of alfalfa on wilt infested soils. J. Amer. Soc. Agron. 27 : 365-366. 1935.
4. Jones, F. R. Testing alfalfa for resistance to bacterial wilt. J. Agr. Res. 48 : 1085-1098. 1934.
5. Jones, F. R. Bacterial wilt of alfalfa and its control. U.S.D.A. Circ. 573, 8 pp. 1940.
6. McKenzie, R. E., D. H. Heinrichs, and L. J. Anderson. Hay and crude protein yields, winter hardiness and wilt resistance of alfalfa varieties. Sci. Agr. 32 : 626-633. 1952.
7. Peake, R. W. Forage crops. *In* Progress Report 1937-1946, Dominion Experimental Station, Lethbridge, Alta., pp. 41-50. 1949.
8. Peake, R. W. Forage crops. *In* Progress Report 1947-1952, Dominion Experimental Station, Lethbridge, Alta., pp. 32-37. 1954.
9. Peltier, G. L. The relative susceptibility of alfalfas to wilt. Nebraska Agr. Expt. Sta. Res. Bull. 66, 16 pp. 1933.
10. Weihing, R. M., and D. W. Robertson. The comparative performance of alfalfa varieties in nursery and field plots in irrigated soil infested with *Phytomonas insidiosa*. J. Amer. Soc. Agron. 35 : 125-136. 1943.

NOTE ON THE GROWTH OF FLOR YEASTS

Flor yeasts are those which, while capable of ordinary fermentation of sugar to alcohol, will also grow as a film or scum on the top of a dry (sugar-free) wine. Desirable strains of the yeast, as a film, impart to wine the flavours and odours of some traditional types of sherry wine. The wine is left undisturbed under the film for months or years to permit absorption of adequate amounts of the desired character from the yeast.

Attempts to make flor-type sherry wine in Canada have usually failed because the yeast did not grow. Similar failure was noted elsewhere (1). This laboratory undertook work on flor yeasts in 1953 at the invitation of the Canadian Wine Institute. While the project is far from completion, it is felt desirable now to place on record certain developments that may be of immediate value to members of the Canadian Wine Institute and others interested in flor yeasts.

Useful amounts of flor character have now been obtained in domestic wine within two to three weeks. In each case the wine was inoculated with 2 per cent by volume of flor yeast, and then subjected to violent agitation. The yeast was freshly cultured on diluted, pasteurized juice of the variety of grape used to make the wine.

Other factors may have contributed to development of flor character. For instance, the addition of 2 per cent by volume of pasteurized grape juice, of like variety, to a wine often hastened development of a film under ordinary (no agitation) circumstances, and it usually had definite utility when the wine was agitated. Provision of a large growth area in the form of a tower filled with glass beads seemed sometimes to aid in quick development of flor character.

Two methods of agitation were used. In the first method, the inoculated wine was placed in small flasks fastened to a mechanically shaken table. The action was continuous during three weeks. In the second method, small centrifugal pumps either moved inoculated wine at half-hour intervals through towers of glass beads or merely re-circulated the wine during about two-minute periods. The action of the pumps was such that the wines were beaten vigorously.

Agitation in closed systems seemed preferable to open systems. The latter sometimes failed to develop flor character in the disturbed wine, even though cell growth was plentiful.

Counts of yeast cells indicated much greater (10 times or more) activity in agitated samples of inoculated wine as compared to similar samples held quietly on a shelf. Sometimes clustering or grouping of cells was concurrent with flor character development but flor character sometimes was evident when the cells were dispersed.

An additional effect of violent agitation was the development of smoothness as an organoleptic character. It occurred whether or not yeasts were present. Probably it contributed to the partial 'aged' character of samples which developed flor odours and flavours.

There is no doubt that physical violence brought about vigorous growth of flor yeast. Flor characteristics developed usually, though not invariably, in inoculated wine that was vigorously agitated. As far as the authors are aware, the use of violent agitation to encourage development of flor character is a novel procedure.

REFERENCE

1. Fornachon, J. C. M. Studies on the sherry flor. Preface, p. vii. Australian Wine Board. 1953.

— R. E. CROWTHER,

— J. H. L. TRUSCOTT,
Horticultural Experiment Station,
Ontario Department of Agriculture,
Vineland Station, Ont.

April 4, 1955.